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IN VIVO AUGMENTATION OF THYROTROPIC HORMONE AND PARTIAL REACTIVATION OF IODINATED (INACTIVE) THYROTROPIC HORMONE EXTRACT BY GOITROGENS[1]

A. ALBERT,[2] RULON W. RAWSON, CHARLOTTE RIDDELL, PRISCILLA MERRILL AND BEATRICE LENNON

From the Thyroid Clinic, Massachusetts General Hospital, and the Department of Medicine, Harvard Medical School

BOSTON, MASSACHUSETTS

IN PREVIOUS studies we have shown that a pituitary extract was rendered incapable of exerting a thyroid stimulating action after in vitro treatment with elementary iodine. The inactivation was readily reversed by reducing compounds both goitrogenic and non-goitrogenic. Furthermore, it was found that the thyrotropic potency of the untreated pituitary extract was enhanced by exposure in vitro to small amounts of goitrogens (Albert and Rawson, 1946; Albert, Rawson, Merrill, Lennon and Riddell, 1946, 1947a, b). From these reactions several interesting possibilities concerning the interaction in vivo of thyroid stimulating hormone and the goitrogens arise. It is our purpose to present experiments indicating that a partial reactivation of iodinated (inactivated) thyrotropic extract and an enhancement of untreated (active) thyrotropic extract can be induced in vivo by goitrogenic compounds.

MATERIALS AND METHODS

The thyroid stimulating hormone (TSH or thyrotropin) extract used, the methods for its iodination, and the method of bio-assay for thyrotropic potency have been previously described in detail (Albert, Rawson, Merrill,

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[2] Present address: Mayo Clinic, Rochester, Minnesota.

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Lennon and Riddell, 1946). For each experiment, four solutions were freshly prepared in 200 ml. quantities. The thyrotropin extract for each experiment was obtained from a fresh vial of the hormonal material. The four solutions were (1) iodinated extract, one ml. containing 400 μg. protein to which 66 μg. iodine were bound, (2) untreated extract, 400 μg. protein per ml., (3) a mixture of extract (400 μg. protein) and KI (66 μg. iodine as iodide) per ml., and (4) 66 μg. iodine as KI per ml. These solutions were administered in 1 ml. amounts subcutaneously once daily for 3 days, and the animals sacrificed on the 4th day, precisely the routine used for the bio-assay of thyrotropic potency. Thyroid weight and thyroidal iodine concentrations were determined by methods previously described.

The day old chicks were received in lots of 200 animals each. On arrival the lot was divided into two groups: one group (A) was placed on a normal

TABLE 1. COMPOSITION OF SOLUTIONS

	Total amounts of agents per chick		Groups	
	Protein (TSH extract)	Iodine	A	B
	μg.	μg.	Subgroups	
Controls	0	0	A_1	B_1
Iodinated thyrotropin	1200	200	A_2	B_2
Thyrotropin	1200	0	A_3	B_3
Thyrotropin and KI	1200	200	A_4	B_4
KI	0	200	A_5	A_5

mash diet and drinking water. The other group (B) was fed the same diet containing 0.2 per cent thiouracil and 0.1 per cent thiouracil in the drinking water (hereafter referred to as a thiouracil diet). Twenty-four hours after the diets were started, each group was divided into five subgroups, consisting of 10–20 animals each. One of these subgroups (A_1 or B_1) on each diet was maintained as a control for its respective group. The other four subgroups (A_2–A_5 or B_2–B_5) were given the solutions in the dosage listed above. The diets were maintained until the 4th day, when the animals were sacrificed. Thus, the plan of the experiment was such that groups of chicks eating a normal diet received by injection nothing (A_1), inactivated thyrotropin (A_2), active thyrotropin (A_3), thyrotropin plus iodide (A_4), and iodide alone (A_5). The respective subgroups (B_1–B_5) of the chicks on thiouracil received the same materials in the same amounts from the same flasks. The composition of the various solutions is listed in Table 1, and inspection of this table reveals the necessity for including iodide and mixtures of iodide and thyrotropin as control materials. Thus, the animals were on thiouracil for a total of four days, during which they received the various solutions on the last three days. Since the group given a normal diet was treated in the same way, the entire experiment thus represented a bio-assay for thyrotropic potency. In this way, the effect of iodinated thyrotropin, thyrotropin, a mixture of iodide and thyrotropin, etc., in chicks eating a normal diet can be compared with controls receiving no injections. The same was true of the chicks on thiouracil diet. Furthermore, the effects of each of these agents could then be compared in chicks on a normal diet and chicks on a goitrogen.

Another goitrogen, 3 phenylaminomethyl-2-merocaptothiazoline (TC-105), was studied by the same plan. The only modification was that on arrival, the group receiving the goitrogen was injected intraperitoneally with 10 mg. TC-105 and then placed on a diet containing 0.2 per cent TC-105. They were not given TC-105 in the drinking water. On the following day injections were begun and the remainder of the experiment was conducted as described above.

RESULTS

The results obtained when thiouracil was employed as the goitrogen are shown in Table 2. The weights of the thyroid glands of the

TABLE 2. EFFECT OF ACTIVE AND INACTIVE TSH ON THE THYROIDS OF CHICKS ON NORMAL AND THIOURACIL DIET

Agents per chick*	Normal diet		Thiouracil diet	
	Thyroid weight mg.	Thyroid I μg. per mg.	Thyroid weight mg.	Thyroid I μg. per mg.
None	4.00	0.78	4.28	1.68
I-TSH†	3.70	0.76	5.80	0.08
TSH	6.50	0.07	9.14	0.00
TSH and KI	6.35	0.34	9.00	0.00
KI	3.70	1.14	4.28	1.25

* 100 chicks total, 10 animals per subgroup. Average body weights varied from 45–50 gm. at end of the experiment.

† I-TSH and TSH are abbreviations respectively for iodinated thyrotropic extract, and active (untreated) thyrotropic extract.

control (uninjected) chicks on a normal diet as compared with those on thiouracil show little difference within four days. The high iodine concentrations also indicate that thiouracil had induced no, or minimal, goiterous changes within this time. Injection of iodinated thyrotropic extract, however, induced a marked difference in the corresponding subgroups. In chicks on the ordinary diet, no goiters were, of course, induced but in chicks fed thiouracil, an appreciable increase in thyroid weight occurred. Animals on thiouracil given active thyrotropin likewise showed larger goiters than those on a normal diet receiving the same dose of thyrotropin. This situation was true when a mixture of active thyrotropin and potassium iodide was given. Iodide alone induced no goiterous changes in either subgroups. The thyroidal iodine concentrations reflected the thyrotropic potency in the same general way as did the thyroid weights; the greater the thyroid stimulating action, the greater the thyroid weights and the lesser the thyroidal iodine concentration.

The extent of these differences can be calculated in quantitative terms. The relationship between thyroid weight and dosage of thyrotropic extract is linear between average weights of 4 and 8 to 9 mg. and the unitage of thyrotropin can be read off from the curve (Albert *et al.* 1946) or can be calculated from the regression equation:

$x = 0.647y - 2.316$ where x = Junkmann-Schoeller units, and y = average weight of the thyroid gland (paired) of 10–20 chicks. The equation yields slightly lower unitage than the curve fitted by eye. Weights above 9.0 mg. can not be used with any degree of accuracy since the dose-response relationship is no longer linear, the curve becoming rapidly asymptotic. A weight of 11 mg. for example, may be obtained with either 3.5 or 50 units of hormonal extract. Because weights of 9 mg. or more represent asymptotic values, all such weights were assigned a minimal value of 3.6 units. This represented a conservative estimation. Calculations[3] made on such conservative estimates will bear a distinguishing plus sign to indicate that their true value could be from 2–10 times higher. A thyroid weight of 4.0 mg. or less was considered as indicative of no thyrotropic stimulation.

Reading off from the curve, or substituting in the equation, it was found that the units corresponding to the respective weights of the glands given in Table 1 were (A) group on the normal diet: 0 for uninjected, 0 for iodinated thyrotropic extract, 1.9 for thyrotropin, 1.8 for the mixture of thyrotropin and KI, and 0 for KI; (B) group on thiouracil diet: 0.5 for uninjected, 1.45 for iodinated thyrotropin, 3.6+ for thyrotropin, 3.6+ for the mixture of thyrotropin and KI, and 0.5 for KI.

Thus, iodinated thyrotropin when given to normal chicks had no activity, but the same amount of iodinated pituitary proteins given to animals fed thiouracil represented 1.45 units. This value when compared with the untreated thyrotropin standard represented 76 per cent reactivation $\frac{(1.45 \times 100).}{1.90}$ However, the controls on thiouracil were allowed 0.5 unit for a weight of 4.28 mg. It was doubtful that this weight represented any thyrotropic effect since the iodine concentration was normal. After subtracting this to allow for the questionable goitrogenic effect of thiouracil under the experimental conditions, the amount of reconversion was 50 per cent $\frac{(1.45 - 0.5 \times 100}{1.90}$ an appreciable difference. The effect of iodinated thyrotropic extract on normal chicks, as stated, was nil but the same dose given to chicks on thiouracil was in terms of units about 300 per cent greater than the thiouracil controls $\frac{(1.45 \times 100).}{0.5}$

The comparative action of active thyrotropin on chicks fed the normal and thiouracil diet was interesting, and showed an augmen-

[3] The calculations consist of determining the percentage difference in units of the experimental values over the standard dose of thyrotropin administered. Such percentage differences are, of course, greater when the standard in units is lower than when the standard is higher. However, lacking a fixed standard such as an International Standard for thyrotropin, this is a convenient, although admittedly inexact, method of handling similar groups of data.

tation by thiouracil of 90 per cent plus $\frac{(3.6\times100).}{1.9}$ The same was true when thyrotropin was given with iodine. Thus, a submaximal dose of hormonal material was more effective in the presence of thiouracil in vivo then in its absence. That augmentation can not be held responsible for what has been termed reactivation can be seen from the following. Assuming that iodinated extract contains 5 per cent hormonal material escaping inactivation during iodination (actually no hormonal activity can be demonstrated), the augmentation of this

TABLE 3. EFFECT OF ACTIVE AND INACTIVE TSH ON THE THYROIDS OF CHICKS ON NORMAL AND TC-105 DIET

Agents per chick*	Normal diet		TC-105 diet	
	Thyroid weight mg.	Thyroid I μg. per mg.	Thyroid weight mg.	Thyroid I μg. per mg.
None	3.2	0.89	4.0	0.28
I-TSH	3.3	0.85	5.2	0.04
TSH	7.2	0.12	10.7	0.003
TSH and KI	5.2	0.30	8.0	0.03
KI	3.4	0.96	3.4	0.24

* 130 animals total, 10–15 chicks per subgroup. Average body weights ranged from 40–47 gm. at completion of experiment.

amount would be equivalent to about 0.2 unit ($1.9\times.05\times2$). Even subtracting this amount $\frac{(1.45-0.5-.2\times100),}{1.90}$ the extent of reactivation would be 40 per cent that of the control thyrotropin given. Consequently, slightly less than half of the original thyrotropic potency after inactivation by exposure to iodine in vitro can be restored by the in-vivo presence of thiouracil.

The same relations were found in the experiment utilizing TC-105 as the goitrogen (Table 3). In this case, the reactivation was 45 per cent $\frac{(1.06\times100).}{2.35}$ The augmentation of active extract with and without added iodide was 54 per cent plus $\frac{(3.6\times100)}{2.35}$ and 160 per cent $\frac{(2.85\times100)}{1.06}$ respectively. It is to be noted, however, that the lowered iodine concentration of the control subgroup (B_1) on TC-105 may indicate a minimal goitrogenic effect.

DISCUSSION

The experiments have shown that the in-vitro reactions reported previously dealing with reactivation of iodinated thyrotropin and the augmentation of active thyrotropin by certain goitrogens can be

duplicated at least partially in vivo. The reactivation in vivo was not as complete as in vitro but the augmentation in vivo was more extensive than in vitro. The probable site at which these reactions may occur in the body was not evident. Perhaps the thyroid, the blood stream, or both, may be considered as possible sites of action.

The results reported here seem to carry some implications for the

TABLE 4. EFFECT OF ACTIVE AND INACTIVE TSH ON THE THYROIDS OF CHICKS AT VARIOUS INTERVALS DURING A NORMAL AND A THIOURACIL DIET

Agents	Normal diet	Thiouracil diet
	Thyroid weight mg.	Thyroid weight mg.
5 days on diet*		
None	4.3	4.3
I-TSH	4.7	5.2
TSH	8.4	9.3
TSH and KI	7.6	9.0
KI	4.5	3.9
10 days on diet		
None	4.9	19.4
I-TSH	4.9	18.4
TSH	9.9	17.2
TSH and KI	9.0	23.2
KI	4.8	15.2
15 days on diet		
None	6.6	30.0
I-TSH	7.8	40.1
TSH	17.0	36.5
TSH and KI	15.0	44.0
KI	8.5	37.4
25 days on diet		
None	12.0	46.4
I-TSH	11.6	53.0
TSH	22.8	55.4
TSH and KI	22.3	83.1
KI	13.2	81.9

* 200 chicks on each interval of diets.

mechanism of action of the goitrogens. As one of the possibilities for the action of substances like thiouracil, the notion that the goitrogen rendered the thyroid more sensitive to endogenous thyrotropin was explored. Astwood (1945) injected thyrotropin into hypophysectomized rats given thiouracil and reported that the goitrogenic action of thyrotropin was not enhanced over that of controls receiving no thiouracil. Because of this and much other data, it was concluded that the goitrogenic action of substances like thiouracil was due to the reflex liberation of increased endogenous thyrotropin occurring in the ensuing myxedematous state of the animals.

Our observations indicate that oral thiouracil and TC-105 which

did not induce goiters in 4 days nevertheless rendered the thyroid markedly sensitive to a standard exogenous dose of thyrotropin. The increase in sensitivity, judging from the resulting goiters, was approximately equivalent to a doubling of the thyrotropic standard. However, this sensitivity can not be demonstrated if the experiment is conducted at a time when thiouracil is inducing a rapid growth of the thyroid. The evidence for this statement was obtained by conducting the experiment in the same fashion as described with the

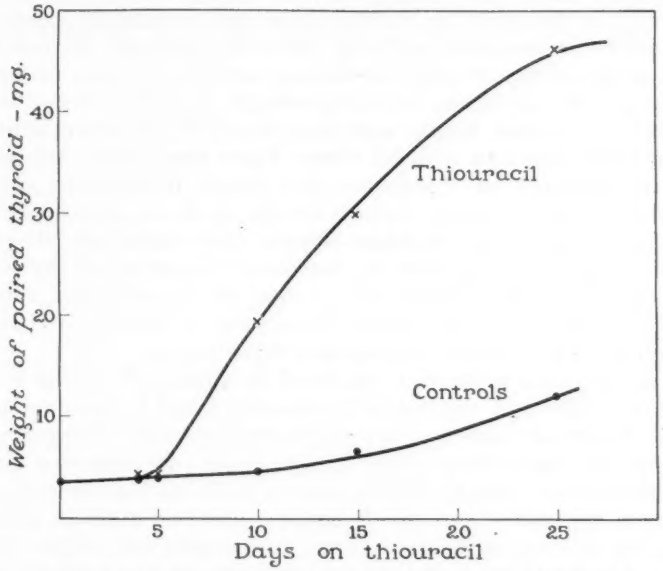

FIG. 1. Relationship between the weight of the thyriod and the length of time during a thiouracil diet as compared with a normal diet.

exception that the three daily injections of the 4 solutions were given so that the experimental chicks were on thiouracil for 5, 10, 15, and 25 days. The effect of the various solutions at the indicated intervals during this regimen is shown in Table 4. It is seen that goiters are rapidly induced somewhere between the 5th and 10th day on the thiouracil diet and the size of the goiters increases at a rapid rate during this interval. Presumably this interval represents a phase during which endogenous thyrotropin is being secreted maximally and the thyroids are responding maximally, a condition which is best illustrated graphically by plotting the size of the goiters against time (Fig. 1). It would appear then, that additional thyrotropin given exogenously during the rapid and asymptotic portions of the growth curve of the thyroid could hardly effect a further increase in the size

of the goiters. Reference to Table 4 will show that the increased sensitivity of the thyroid as described by our experimental procedure is lost at the 10th day of thiouracil therapy. Taking thyrotropin alone, it is seen that at each interval in therapy, good thyrotropic action was induced in chicks on a normal diet but no difference was noted when the same dosage of thyrotropin was given to chicks on a thiouracil diet as compared with their controls. This was also true of iodinated thyrotropin which was inactive when given to normal chicks at various ages. The same dose of iodinated thyrotropin, however, in the chicks on thiouracil induced no change in the size of the goiters as compared with the thiouracil controls. Even if complete reactivation occurred, no change would be evident because an equivalent dosage of untreated thyrotropin had no further effect on the goiters. Similar results were obtained with the thyrotropin and KI mixture, and also with KI alone. There were some variations in the size of goiters, but it is known that the size of the goiter in chicks maintained on thiouracil alone is erratic when an asymptotic level is reached. It would therefore appear that while the thyroid is being maximally stimulated by increased quantities of thyrotropic hormone liberated endogenously during the hypothyroid state induced by thiouracil, no further stimulation of the thyroid can be expected by the addition of exogenous thyrotropin.

The increased sensitivity conferred by thiouracil on the responsiveness of the chick thyroid to thyrotropin would seem in opposition to the results obtained on hypophysectomized rats. However, they may not be unalterably opposed because of two unknown factors, one pertaining to the chick work and the other, to the rat work.

First, our chicks were not hypophysectomized animals, and consequently we can only surmise from the weights and iodine concentrations of the thyroids that the chick pituitary was liberating little thyrotropin during the first four days of its life or while on a thiouracil diet during its first four days of life. In any case, the use of nonhypophysectomized chicks is not as rigorous an experimental procedure as the use of the hypophysectomized rat, and therefore our results can not be strictly compared with those of Astwood. The second point pertains to the dosage of thyrotropin employed in the rat work. Since there are few data on dose-response relationships between thyrotropin and thyroid weight in the hypophysectomized rat, it is not known with certainty whether increased sensitivity could not be demonstrated with dose levels of thyrotropin other than that used. If our results on chicks can in any way be applied to the rat, it would appear that increased sensitivity might occur with submaximal doses, but perhaps not with maximal or minimal doses. Pending further information on both factors, it is not possible to state now that the chick and the rat thyroid are affected differently by thiouracil as regards sensitivity to thyrotropin.

SUMMARY

The administration of a standard amount of thyrotropic hormone extract to chicks receiving thiouracil or 3-phenylaminomethyl 2-mercaptothiazoline in the diet resulted in a marked augmentation of thyrotropic potency when compared with controls receiving no goitrogen. Administration of iodinated thyrotropic extract which was inactive when given to control chicks resulted in appreciable thyrotropic action in chicks receiving either of the two goitrogens orally. The augmentation induced was approximately 100% whereas the reactivation approximated 50% that of the hormonal extract prior to inactivation by iodine.

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AN IMPROVED METHOD OF EXTRACTION AND PURIFICATION OF RELAXIN FROM FRESH WHOLE OVARIES OF THE SOW[1]

A. ALBERT,[2] W. L. MONEY AND M. X. ZARROW
From the Biological Laboratories, Harvard University
CAMBRIDGE, MASSACHUSETTS

RELAXIN, a water-soluble, physiologically active substance of the ovary has been prepared in partly purified form by two different methods. Fevold, Hisaw, and Meyer (1932) extracted fresh sow corpora lutea with acid alcohol and worked up the active material by solution in and precipitation from organic solvents. The activity of the final product, prepared as an iso-electric precipitate at pH 5.4, was 30 units per mg. Using semi-dried, defatted luteal tissue obtained as a by-product from a commercial procedure for the preparation of progestin, Albert, Money, and Zarrow (1946) prepared relaxin with approximately the same order of activity by differential solubility in water, ammonium sulfate solutions and cold dilute alcohol.

The purpose of this paper is to present an improved method for the extraction and purification of relaxin from fresh whole ovaries of the sow. The proposed method offers the advantage over the two previous methods in that a product ten times more active can be obtained with only three main steps. The increase in potency and the ease of preparation were due partly to the methods employed and partly to the use of fresh whole ovaries which were found to contain appreciably more relaxin activity than either fresh corpora lutea or the defatted luteal tissue residue.

RESULTS

1. *Potency of Ovarian Tissue*

Acid alcohol extracts of fresh corpora lutea were reported by Hisaw (1929) to contain 1 unit of relaxin activity per gram equivalent (gm. eq.) of tissue. After preliminary purification by alcohol, Fevold, Hisaw, and Meyer (1932) reported an activity of 1 unit per gm. eq. of fresh corpora lutea but the overall yield at this point of their procedure was not stated. Using corpora lutea residue, Albert, Money, and Zarrow (1946) found acid aqueous extracts to contain 3–4 units per gm. residue, which corresponded to a relaxin content of about 1

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[2] Present address: Mayo Clinic, Rochester, Minnesota.

unit per gm. eq. of the original fresh corpora from which the residue was prepared as a by-product.

However, acid aqueous extracts[3] of unselected fresh whole ovaries[4] in five batches ranging from 1 to 10 kg. yielded activities of 200, 100, 200, 300, and 400 units[5] per gm. eq. Three possibilities to account for this large discrepancy in potency were considered: (a) that previous methods of extraction were inefficient, (b) that corpora lutea contained only part of the active material, (c) that the potency of ovarian tissue was extremely variable.

2. *Efficacy of Methods of Extraction*

Two kg. fresh unselected whole ovaries were ground to a mash, and 0.5 kg. aliquots of the mash extracted according to the two previously published methods, and according to the method described (footnote 3). In each case, after the combined extract (first extract) was removed for assay, the residue was re-extracted (second extract) by the method described (footnote 3). The activities of the first and second extract are given in Table I. The method of Fevold, Hisaw and Meyer (1932) which yielded 1 unit/gm. eq. of corpora lutea efficiently extracted most of the activity (200 units/gm. eq.) of fresh whole ovarian tissue. By re-extracting the residue (second extract) only a small amount of activity could be further obtained. Similarly, the method of Albert, Money, and Zarrow (1946) in which the ovarian tissue was first washed with methanol to remove luteal steroids removed none of the relaxin activity (first extract). Extraction of the residue yielded full activity (second extract), as previously reported. Thus, the efficiency of the procedures previously reported were confirmed, and consequently, the low potency reported previously cannot be ascribed to inefficiency of extraction methods.

TABLE I. COMPARISON OF EFFICACY OF METHODS OF EXTRACTION

First extract	Units/gm. eq.	Second extract	Units/gm. eq.
Acid alcohol (Fevold, Hisaw, Meyer)	200	As in footnote 3	25
Methanol (Albert, Money, Zarrow)	0	As in footnote 3	200
As in footnote 1	200	As in footnote 3	25

[3] The extract was essentially the same as reported by us for luteal residue. The ovaries were ground, extracted with 4 weight volumes of 2 per cent HCl for one day, adjusted to 1 M NaCl and pH 6.5, and filtered. The residue was re-extracted in the same fashion and the united filtrates assayed.

[4] The high activity of fresh whole ovaries was first found by Dr. Robert L. Kroc, Research Laboratories, Maltine Company, N. Y., who kindly placed this information at our disposal.

[5] The method of assay and comparison of units are given in our previous report.

3. *Distribution of Relaxin in the Ovary*

One-half of a 2 kg. batch of whole fresh unselected ovaries was extracted as described (footnote 3) and the other half separated by dissection into three main portions: corpora lutea, follicular fluid and remainder of ovary (stroma, follicles, etc.). These portions were extracted separately (footnote 3) and assayed (Table II). The result showed that the physiologically active substance inducing relaxation

TABLE II. DISTRIBUTION OF RELAXIN IN THE OVARY
(unselected whole ovary)

Tissue	Units/gm. eq.
Whole ovary	200
Corpora lutea	200
Follicular fluid	0
Remainder of ovary	200

TABLE III. RELAXIN ACTIVITY OF OVARY IN THE PREGNANT AND NON-PREGNANT SOW

Activity—Units/gm. eq.	
Pregnant	Non-pregnant
3200	5
3000	5
7200	5
7200	5
7200	5
5500	3
9600	8
6000	10
6000	5

was distributed throughout ovarian tissue and was not confined to corpora lutea.

4. *Variability in Potency of Ovaries*

Hitherto only unselected whole ovaries were employed. It was considered that the reproductive state of the sow might influence the relaxin content of the ovary. Consequently ovaries from pregnant and non-pregnant sows were extracted. The results (Table III) showed that the relaxative potency depended on the state of the reproductive system since ovaries from pregnant animals were several hundred times as potent as those from non-pregnant animals. This finding and the finding that relaxin in unselected whole ovaries was not confined to the luteal bodies probably explain the low potency reported previously, if one assumes that the tissue used by previous authors represented corpora lutea chiefly from non-pregnant animals.

5. *Method of Purification*

The following procedure for obtaining relaxin in purified form was developed using fresh whole unselected ovaries. Conceivably, ovaries from pregnant animals might yield even more potent preparations but it is difficult to obtain such tissue in sufficient quantity. The method has yielded relaxin in amorphous state with an activity some ten times greater than has been hitherto reported. Five different preparations assayed 300, 200, 400, 500, and 100 units per mg. respectively. Preparations assaying at 300 to 500 units per mg. appeared to be in a high state of chemical purity.

TABLE IV. ONE KILOGRAM UNSELECTED FRESH WHOLE OVARIES

Steps	Total solid	Units/mg.	Per cent yield-activity
1. Acid extract	8.0	30	100
2. Eluate	2.3	60	57
3. Iso-electric precipitate	0.23	300	29

Step 1: One kg. of fresh sow ovaries was ground to a mash and dispersed into 4 liters of 2 per cent HCL. After extraction for 1 day 160 gm. NaCl was added and the pH of the mixture adjusted to 3.9 with 10 per cent NaOH. The extract was rapidly filtered by gravity and the tissue pulp re-extracted in the same fashion and then discarded. The combined extract was then adjusted to pH 6.5 with 10 per cent NaOH.

Step 2: A suspension[6] of bentonite (80 gm. in 800 ml. 1 per cent KCl) was stirred into the extract for 15 minutes and the clay allowed to settle. The supernatant fluid was decanted. The bentonite was collected and washed twice for 15 minutes each with 200 ml. portions of 6 per cent pyridine brought to pH 6.5 with HCl. The eluate was freed from pyridine by dialysis.

Step 3: The dialyzed solution was brought to pH 4.4 with 3NHCl and kept at 5° C. overnight. The precipitate thus formed was collected by centrifugation and suspended in 100 ml. water at pH 7.0. After removal of insoluble material, the active substance was again precipitated by acidification to pH 4.4. The distribution of activity in one batch during this procedure is given in Table IV.

SUMMARY

The relaxin content of fresh whole ovaries was found to be several hundred times greater than that reported for fresh or defatted corpora lutea.

Ovaries from pregnant sows contained between 500 and 1500

[6] This adsorption procedure was modified from a method reported by Ward, Alderton and Fevold (1945).

times more relaxin activity than ovaries from non-pregnant animals.

Using unselected fresh whole ovaries, a simplified method for the preparation of relaxin with an activity ten times greater than previously reported is described.

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EFFECTS OF TESTOSTERONE PROPIONATE ON BODY WEIGHT AND URINARY NITROGEN EXCRETION OF NORMAL AND HYPOPHYSECTOMIZED RATS[1]

GILBERT S. GORDAN, HERBERT M. EVANS, AND MIRIAM E. SIMPSON

From the Institute of Experimental Biology, and the Division of Medicine, University of California

BERKELEY AND SAN FRANCISCO, CALIFORNIA

IN A PREVIOUS paper (Simpson, Marx, Becks and Evans 1943) it was suggested that the well-known effect of testosterone propionate on body weight might be mediated through the pituitary. This hypothesis was supported by the failure of testosterone propionate to produce a significant increase in body weight and skeletal dimensions in hypophysectomized, young, male rats.

On the other hand, Leonard (1943) reported partial maintenance of weight in hypophysectomized male rats when he injected 10–12 mg. of testosterone over a 10-day period, whereas the controls lost weight. Tyslowitz (1943) has also noted acceleration of growth in hypophysectomized rats. Kochakian (1943) induced nitrogen retention in an hypophysectomized, castrated dog with 25 mg. of testosterone propionate per day.

Furthermore, the growth and nitrogen-retaining effects of testosterone in panhypopituitary dwarfs were reported in 1942 by Howard, Wilkins, and Fleischmann, and in Simmonds' disease by Werner and West (1943). Weight gain in patients whose pituitaries were destroyed by tumor or trauma has also been reported by Escamilla and Lisser (1942) and Lisser and Curtis (1945).

In order to determine whether the anabolic effects of testosterone are mediated by the pituitary, we have compared the effects of testosterone propionate upon body weight and nitrogen excretion of normal and hypophysectomized female rats.

METHODS

Adult female rats aged eight to nine months were selected, because their weight is normally "plateaued" at this age, and a stable base line of weight and nitrogen excretion can thus be obtained. Eighteen animals weighing 235 to 320 gm. were hypophysectomized (the completeness of hypophysectomy subsequently being checked at autopsy by use of the binocular microscope). The animals were then placed in individual screen-bottomed cages and urines

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were collected through ribbed glass funnels into Erlenmeyer flasks. 1-N sulfuric acid was used as a preservative. Feces and hair were collected in the funnels upon perforated porcelain discs. Funnels were washed down and urines collected at 48 hour intervals except during injection periods when urines were collected at 24 hour intervals. Urines were analyzed for nitrogen by the micro-Kjeldahl method.

The animals were kept in a room with a constant temperature of $25° \pm 2°$C.

A purified diet[2] of constant nitrogen content, supplemented by all the known food requirements, was used. In these experiments both hypophysectomized and intact animals were fed an iso-caloric intake of 10 gm. per animal per day. It has been found by trial and error that all of the hypophysectomized animals could be relied upon to eat 10 grams of diet daily (weighed within 0.1 gram). (Intact animals of the same age and weight normally can be relied upon to eat 12 grams of this diet daily.)

The animals were weighed at the same time daily, immediately before feeding. This procedure insured a stable base line of weight.

EFFECTS OF TESTOSTERONE PROPIONATE ON HYPOPHYSECTOMIZED RATS

The hypophysectomized animals were divided into two equal groups, and following a control period of 10 days (starting one week after hypophysectomy), each animal of one group received a daily injection of testosterone propionate 0.5 mg. in 0.1 cc. of sesame oil subcutaneously, while each animal of the other group received a control injection of 0.1 cc. of sesame oil.

By reference to Table I it will be seen that when 0.5 mg. of testosterone propionate was injected daily, the urinary nitrogen excretion of the testosterone injected animals showed a slight decrease, but not a significant one, while the average weights of both experimental and control groups fluctuated but one gram. This was considered a negative result, and the dose was therefore increased to 2.5 mg. of testosterone propionate in 0.5 cc. of sesame oil daily. The control animals received 0.5 cc. of sesame oil per day. With this dose, the average daily nitrogen excretion of the testosterone injected animals fell to 180 ± 5.7 mg. while the controls maintained an average daily nitrogen excretion of 240 ± 4.1 mg. When the difference, amounting to a retention of 60 mg. per animal per day is calculated as protein plus protein-bound water, it is more than adequate to account for the slight weight gain of 4 grams (range -1 to $+7$ grams). ($0.060 \times 6.25 = .375$ gm. protein; $.375 \times 5 = 1.87$ gm. tissue $\times 5$ days $= 9.4$ gm. calculated

[2] The diet consisted of alcohol-extracted casein 24%, sucrose 63.5%, hydrogenated vegetable oil (Crisco) 8%, salts (Hegsted *et al.*) 4%, and liver fraction powder 0.5%. Crystalline vitamins are added: thiamine chloride 5 mg., pyridoxine hydrochloride 5 mg., riboflavin 10 mg., p-aminobenzoic acid 10 mg., nicotinic acid 20 mg., calcium pantothenate 50 mg., inositol 400 mg., choline chloride 1 gm., and 2 methyl 1,4 naphthoquionone (menadione) 5 mg. per kgm. diet. One cc. of a fat-soluble vitamin mixture containing 6 mg. alpha-tocopherol, 115 chick units vitamin D, 800 U.S.P. units vitamin A, and 650 mg. corn oil (Mazola) was furnished weekly, one seventh of the amount being added to the diet daily. The latter procedure was adopted to avoid increments of weight incident to the addition of 1 cc. of oil.

body weight increase). The controls remained stable at 247 grams with an average fluctuation of zero (range −9 to +4 grams). These data are presented graphically in Figure 1.

It will be observed that the effect upon the urinary nitrogen excretion persisted throughout the 10 day post-injection period, and

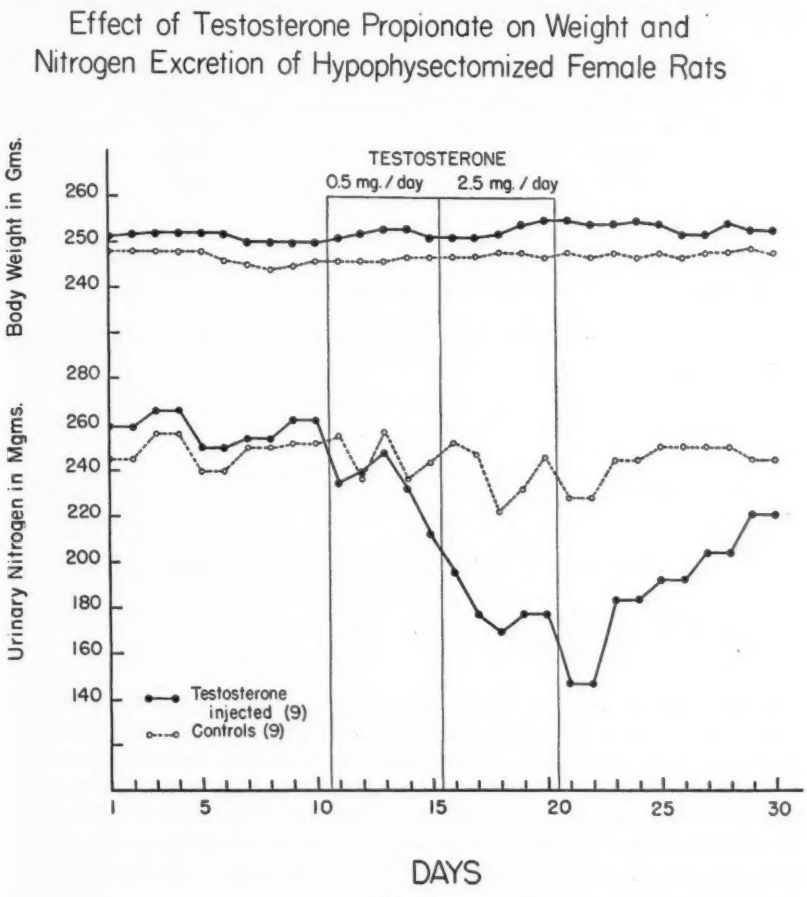

FIG. 1

that the urinary nitrogen excretion had not yet returned to pre-injection levels on the 10th day. This phenomenon is also apparent in the graph of Kenyon *et al.* (1940) and may be in part related to the steroid nature of the hormone and its oily solvent.

EFFECTS OF TESTOSTERONE PROPIONATE ON NORMAL RATS

In Table II can be seen the results of a similar experiment with 10 intact female rats of the same age and weight as the previous group at the time of hypophysectomy. These rats also were fed 10 grams of

diet daily. All other details of the experiment were identical with those in which hypophysectomized rats were used. It will be observed that the urinary nitrogen excretion was somewhat higher than that of the hypophysectomized animals, which may suggest better intestinal absorption of the diet in the intact animals (Althausen, 1943).

It will be observed that when 0.5 mg. of testosterone propionate

EFFECT OF TESTOSTERONE PROPIONATE ON WEIGHT AND AVERAGE DAILY NITROGEN EXCRETION OF ADULT FEMALE RATS ON ISO-CALORIC DIETS

TABLE I. HYPOPHYSECTOMIZED RATS

	10 days pre-injection period	5 days 0.5 mg. injection period	Increment of Wt and N retention	5 days 2.5 mg. injection period	Increment of Wt and N retention	10 days post-injection period
Controls (9 rats) Wt N	246 gm. 248±4.2 mg.*	247 gm. 246±4.6 mg.	+1 gm.	247 gm. 240±4.1 mg.	0	248 gm. 244±5.1 mg.
Testosterone Injected (9 rats) Wt N	250 gm. 258±4.0 mg.	251 mg. 234±6.7 mg.	+1 gm. 12 mg. p†=0.15	255 gm. 180±5.7 mg.	+4 gm. 60 mg. p=<0.01	253 gm. (last 48 hrs.) 221±13.1 mg.

TABLE II: INTACT RATS

Controls (5 rats) Wt N	269 gm. 268±3.3 mg.	269 gm. 270±3.7 mg.	0	269 gm. 281±4.9 mg.	0	269 gm. 286±3.3 mg.
Testosterone Injected (5 rats) Wt N	259 gm. 266±5.5 mg.	269 gm. 234±5.9 mg.	+10 gm. 36 mg. p=<0.01	276 gm. 222±8.8 mg.	+7 gm. 59 mg. p=<0.01	275 gm. 269±5.1 mg

Wt = average body weight at end of period.
N = average daily nitrogen excretion.
* = standard deviation of the mean.
† = Fisher's probability value.

was injected daily, the intact animals showed a somewhat greater degree of nitrogen retention than did the hypophysectomized animals receiving the same dose. More striking, however, was the marked increase in weight: 10 grams per animal in five days (range +7 to +14). This is considerably greater than the theoretical weight gain calculated from the amount of nitrogen retained (.035×6.25=.218 gm. protein×5=1.09 gm. tissue×5 days=5.4 gm. calculated body weight increase).

When the dose of testosterone propionate was increased to 2.5 mg. per day, the urinary nitrogen excretion decreased further. It is to be noted that the amount of nitrogen retained at this dose level was the same in hypophysectomized and intact animals. The weight of the intact animals continued to increase strikingly, and to a greater degree than can be explained by the amount of nitrogen retained. The total gain in 10 days amounted to 17 grams per animal (range +13 to +22), a gain such as might be produced with hypophyseal growth hormone. These data are presented graphically in Figure 2.

Since the increase in weight of the intact animals exceeded that which could be calculated from the amount of nitrogen retained, it seems likely that the increase was, at least in part, due to water storage. That testosterone can produce water retention, and even edema, is well known.

The hypophysectomized animals, however, gained but little

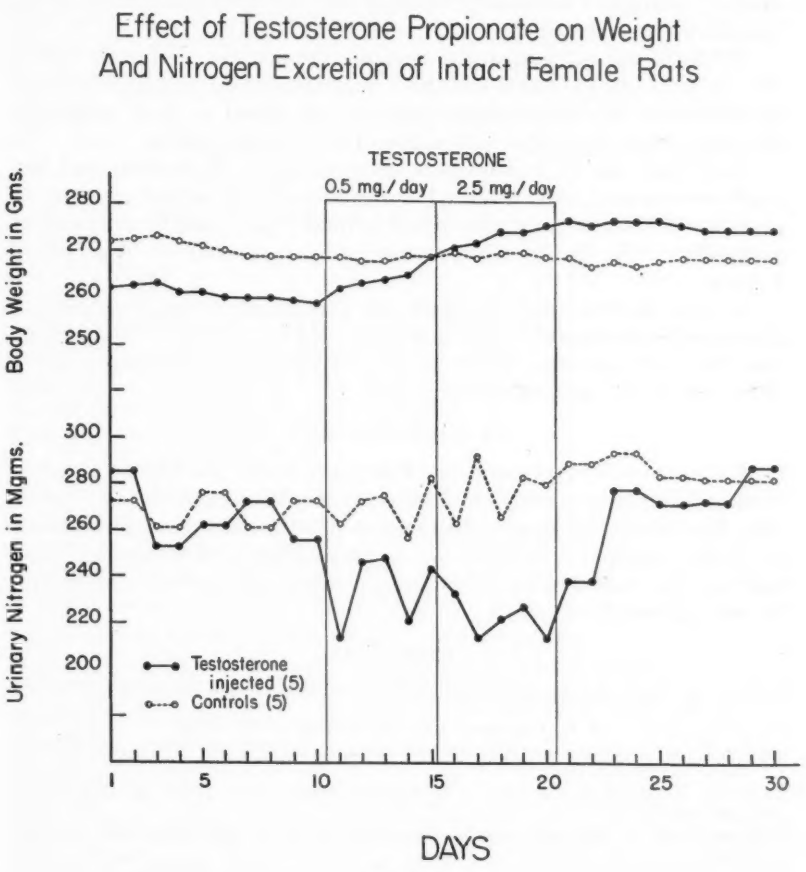

FIG. 2

weight; indeed, less than might be anticipated from the amount of nitrogen retained. From these data there was thus no evidence of water retention in these animals. One might wonder if the water retaining effect of testosterone requires the presence of the antidiuretic principle of the posterior lobe.

In a recent paper Page, Ogden and Anderson (1946) concluded that testosterone produced toxic effects in hypophysectomized rats.

No such toxicity was found in our animals upon either dose tested. However, their rats also had renal hypertension, and the discrepancy may therefore be attributed to the difference in experimental conditions.

SUMMARY

The effects of testosterone propionate upon body weight and urinary nitrogen excretion of normal and hypophysectomized adult, female rats were studied.

With 0.5 mg. of testosterone propionate per day, but little reduction of nitrogen excretion occurred in both normal and hypophysectomized rats. The hypophysectomized rats failed to gain weight on this dose while the intact rats gained 10 grams in 5 days.

With 2.5 mg. of testosterone propionate, both normal and hypophysectomized rats retained a significant and equal amount of nitrogen. With this dose, the intact animals continued to gain weight markedly, while the hypophysectomized rats gained but 4 grams in 5 days.

It thus appears that testosterone propionate produces an equal amount of nitrogen retention in normal and hypophysectomized rats, but that utilization of the retained nitrogen to form body weight differs markedly in these two groups.

ACKNOWLEDGEMENTS

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ADAPTATION OF THE SPIRAL ARTERY IN THE RABBIT OVARY TO CHANGES IN ORGAN-SIZE AFTER STIMULATION BY GONADOTROPHINS; EFFECT OF OVULATION AND LUTEINIZATION

S. R. M. REYNOLDS

Department of Embryology, Carnegie Institution of Washington
BALTIMORE 5, MARYLAND

UNTIL RECENTLY, scant attention has been paid to the arrangement of the arteries within the ovary. Older works, such as those of Barkow (1866) and Clark (1900) depict them in the human as a mass of tortuous vessels derived from branches of the ovarian artery as this vessel enters the hilus of the ovary. Later work, based upon gross dissections in the rat, guinea pig, sow, and rabbit (Anders, 1927) do not alter this conception. This work shows schematically the arteries in the hilus of the ovary as forming a network derived from several arteries in the mesovarium.

A different description is given by Belou (1934). This investigator examined by stereoscopic X-ray the human female genital tract in which the arteries had been injected with an opaque medium. In the legend of one picture, this author notes that in the ovary shown one may see twisted masses of vessels arising from the helical blood vessel in the hilus of the ovary. Unfortunately, the picture and the description lack enough detail to enable the reader to appreciate the nature of the structure to which reference is made although they do suggest that Belou may have been the first investigator to see the spiral arrangement of the artery in the hilus.

More recent observations, based upon injected preparations of the rat ovary, indicate that as the ovarian artery enters the hilus it "divides into several secondary trunks which penetrate the medulla. These coil and twist to a marked degree as they pass toward the cortical zones. From these, smaller branches are given off to the medullary stroma whereas the larger branches supply the cortical region" (Bassett, 1943). For a general review of the literature of this subject, the reader is referred to the paper by Bassett.

In contrast to the above general concept, a recent demonstration in the ovary of the rabbit by means of vinylite plastic injection-corrosion preparations reveals an orderly spiral arrangement of the artery within the ovary (Reynolds, 1947). As the artery enters the

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hilus near the caudal pole of the ovary, it turns sharply cephalad and forms a helix of diminishing diameter throughout the length of the hilus. Terminal arteries to the stroma of the ovary arise from the coils along the length of the spiral. A secondary spiral may arise at the base of the main one and pass caudad. Both spirals are coiled in a counter-clockwise direction. This is the usual pattern. In one instance, among nearly one hundred ovaries examined, two distinct branches of the ovarian artery were seen to enter the hilus of the ovary. Both branches give rise to separate major spiral arteries. This preparation is shown in figure 1, plate I. The venous network in the ovary resembles the pattern described for arteries by Bassett (vide supra). An example of the venous arrangement was shown in a previous communication (Reynolds, 1947), and will be seen in figures 4, 5, and 8 of this paper.

The functions subserved by this special arterial arrangement in the ovary are not known, although two speculations have been advanced with respect to them (Reynolds, 1947). It has been suggested, first, that the arterial arrangement is related to cyclic ovarian growth affording a means of "paying out" of the coils of the artery in response to generalized ovarian growth such as occurs with ovulation or luteinization. Second, it has been suggested that it serves to effect a lowering and equalization of arterial blood pressure throughout the stroma of the ovary by means of established hemodynamic mechanisms which should apply to coiled vessels of diminishing diameter. By exerting this effect locally within the ovary, a mechanism is provided by which the blood pressure is regulated peripherally within the organ regardless of its position as this is shifted during the later

PLATE I

FIG. 1a. Fresh ovary[1] from an isolated rabbit containing large, clear follicles. ×2. Caudal pole at right.

FIG. 1b. Vinylite plastic cast of spiral arteries in the ovary shown in 1a. The two separate arteries from the ovarian artery are exceptional (see text). ×2. Caudal pole at right.

FIG. 2. Spiral artery from a non-pregnant rabbit, recently pseudopregnant. ×2. Caudal pole at right.

FIG. 3a. Fresh ovary, 24 hours after gonadotrophin. Four ovulation points, six large clear follicles, several small fresh corpora hemorrhagica. ×2. Caudal pole at left.

FIG. 3b. Spiral arteries in hilus of ovary shown in 3a. Note beginning extension of coils at arrows. ×3. Caudal pole at left.

FIG. 4a. Fresh ovary, 48 hours after gonadotrophins. Four or 5 new ovulation points. ×2. Caudal pole at right.

FIG. 4b. Injection-corrosion preparation of arteries and veins of ovary shown in 4a. Note paying out of the first two coils (arrow) of the spiral artery in the hilus of the ovary. Several veins drain ovary at cephalic end. ×3. Caudal pole at right.

FIG. 5. Injection corrosion preparation of veins and arteries of an ovary 72 hours after gonadotrophins. Note extreme paying out of the spiral artery so that it is sinuous, or undulating in character. Ovary had 5 corpora lutea and many tiny corpora hemorrhagica. ×3. Caudal pole at left. Two veins drain ovary at the middle and at the cephalic end.

[1] All pictures of fresh ovaries were after the injection of vinyl acetate.

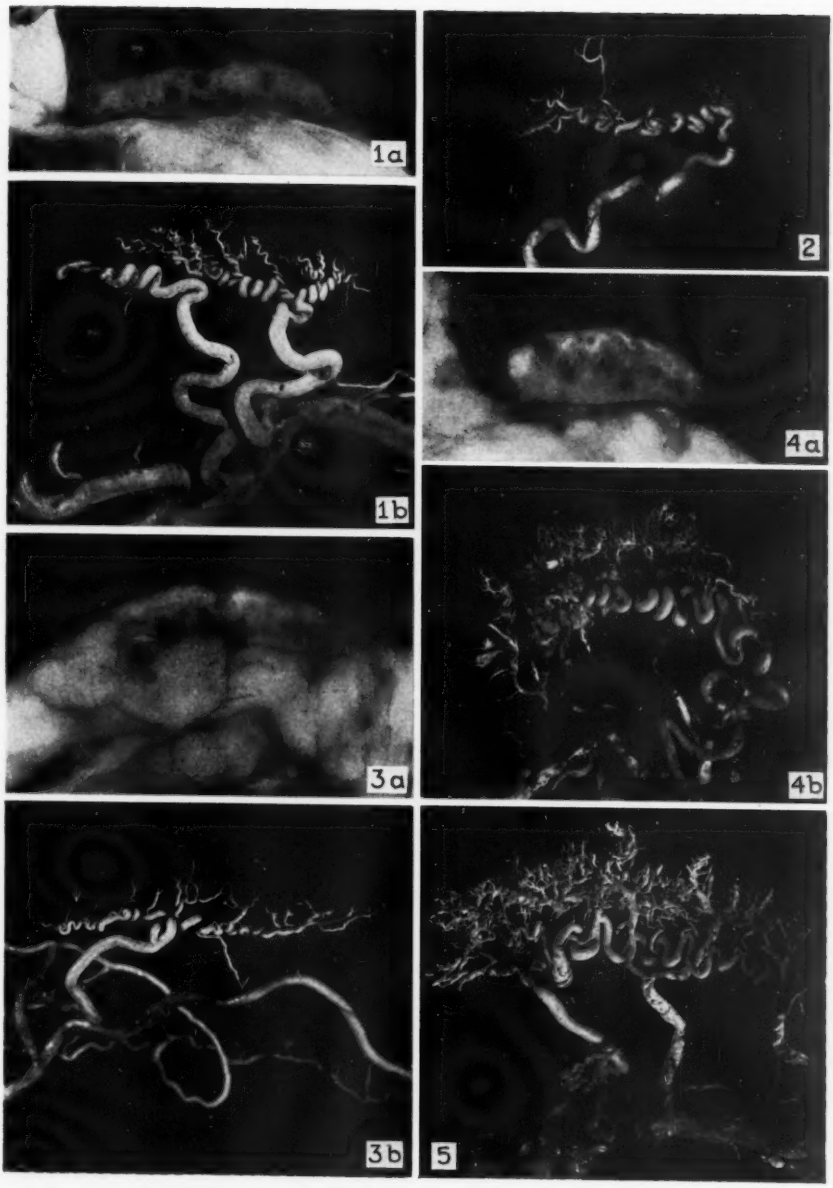

PLATE I

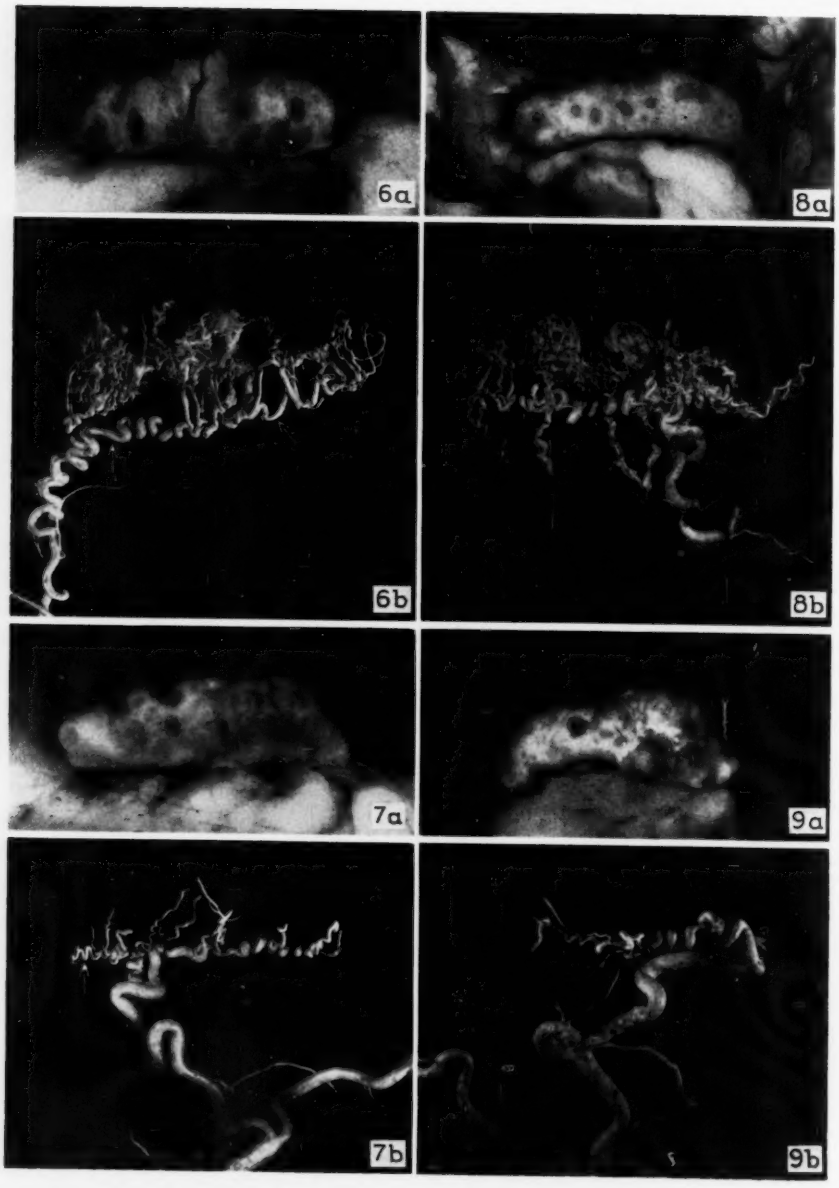

PLATE II

stages of pregnancy. Finally, it has been suggested that abnormal relations with respect to the above two mechanisms may be concerned with ovarian pathology in conditions whose etiology is now unknown. The present report is offered as evidence that the first of the foregoing speculations is correct.

MATERIALS AND METHODS

The effect of growth of the Graafian follicles and of luteinization upon the spiral artery was demonstrated in mature, isolated female rabbits. Stimulation of the ovary was obtained by intravenous injection of the following gonadotrophic substances: Gonadogen (Upjohn), twenty Upjohn units per injection; Pranturon, (Schering), 500 international units per injection; urinary gonadotrophic substance (Abbott), twenty rat units intravenously per injection.

Injections were made at ten o'clock in the morning, in groups of four rabbits each. At twenty-four hour intervals, animals were sacrificed, and vinylite was injected according to the technic described in the original paper on the ovarian spiral artery (Reynolds, 1947). The only modification was that prior to digestion of the soft tissues, photographs of the fresh ovaries were made at a twofold magnification in all but two instances in order to permit orientation of the casts after their preparation with respect to the external form of the ovary.

After clearing away the soft tissue from the vinylite by digestion, stereoscopic photographs were made of the casts for detailed, three-dimensional study. This was done by, and at the suggestion of, Mr. Chester F. Reather, photographer in this department. Unfortunately, the stereographs cannot be made available in this form to the general reader. The change in configuration of the positions of the spiral vessels is shown exquisitely in these pictures, and many important details are lost in the ordinary two-dimensional pictures shown in plates I and II. Careful study of all preparations showed that they could be divided into two groups. These were, a) those in which ovulation, luteinization, and generalized, but not excessive, follicular growth

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PLATE II

FIG. 6a. Fresh ovary[1] 96 hours after gonadotrophins. Four corpora lutea and 8 or more large clear follicles. ×2. Caudal pole at left.

FIG. 6b. Spiral artery of ovary shown in 6a. Note extended condition of the coils (arrows). ×3. Caudal pole at left. Venous network has been partially removed to show spiral artery.

FIG. 7a. Fresh ovary 6 days after gonadotrophins. Four corpora lutea, several clear follicles and small corpora hemorrhagica. ×2. Caudal pole at right.

FIG. 7b. Spiral artery of ovary shown in 7a. Note extended position of coils (arrow) and lateral flattening (best seen stereoscopically). ×3. Caudal pole at left.

FIG. 8a. Fresh ovary 9 days after gonadotrophin. Four corpora lutea, 6 large, clear follicles, several small corpora hemorrhagica. ×2. Caudal pole at right.

FIG. 8b. Spiral artery (and veins) of ovary shown in 8a. Note regular but extended position of coils. ×3. Caudal pole at right.

FIG. 9a. Fresh ovary 12 days after gonadotrophin. Four corpora lutea, 8 medium sized clear follicles. ×2. Caudal pole at right.

FIG. 9b. Spiral artery of ovary shown in 9a. Note relatively regular, but extended position of coils. They are also flattened laterally. ×2. Caudal pole at right.

[1] All pictures of fresh ovaries were after the injection of vinyl acetate.

took place; and b) those associated with exaggerated ovarian growth responses. The latter consisted chiefly of multiple large follicles and large corpus hemorrhagicum cysts. The changes in the spiral arteries of the first group are presented in this paper.

TABLE 1. SUMMARY OF CHANGES IN SPIRAL ARTERY CONFIGURATION IN THE OVARY ASSOCIATED WITH OVULATION AND LUTEINIZATION

Time after Gonadotrophins	Condition of Ovaries		Condition of Spiral Artery	
	Left	Right	Left	Right
0 hrs. (see figure 2)	medium and large follicles (at end of pseudopregnancy)	large and medium follicles	tightly coiled flattened laterally	tightly coiled, flattened laterally
0 hrs. (see figure 1)	many small follicles	many small follicles; very long ovary	regularly coiled	two main spirals, well formed
24 hrs. (see figure 3)	3 ovulation points, many small follicles	2 ovulation points, many small follicles	coiled but somewhat extended and flattened laterally; many terminal branches	coiled but extended and flattened laterally; many terminal branches
24 hrs.	4 ovulation points; few small corpora hemorrhagica	4 ovulation points; 6 large follicles; several small corpora hemorrhagica.	coils "paid out," or extended	coils well formed, numerous terminal arteries
48 hrs. (see figure 4)	4–5 ovulation points	3 new corpora lutea; 1 large corpus hemorrhagicum	coils "paid out" in first two loops, rest well coiled	(to be described later)
72 hrs. (see figure 5)	2 corpora lutea; 3 corpus hemorrhagicum cysts.	5 corpora lutea; many small corpora hemorrhagica	(to be described later)	coils regular but paid out longitudinally, giving undulating appearance.
96 hrs. (see figure 6)	4 corpora lutea; 8 or more large follicles	6 corpora lutea; 2 corpus hemorrhagicum cysts and many large follicles	regular coils much enlarged, but two loops still quite extended.	(to be described later)
96 hrs.	several corpora lutea; several corpora hemorrhagica	several corpora lutea at one end; corpora hemorrhagica at other end of ovary	(to be described later)	(to be described later)
6 days (see figure 7)	4 corpora lutea; few small corpora hemorrhagica	4 corpora lutea; 6 large clear follicles; several small corpora hemorrhagica	first loop straightened out; coil flattened laterally, small loops extended on caudal spiral	regularly coiled flattened laterally at tip
6 days	numerous corpora hemorrhagica only	numerous corpora hemorrhagica only	(to be described later)	(to be described later)
9 days (see figure 8)	4 corpora lutea; 4 corpora hemorrhagica, many medium and large sized follicles	4 corpora lutea; 6 large clear follicles, many small corpora hemorrhagica	coils regular but flattened laterally a little	first two coils extended. Rest regularly coiled
12 days (see figure 9)	2 corpora lutea; 8 medium sized follicles	3 or 4 corpora lutea; 4 medium to large sized follicles	regularly coiled, but flattened laterally	(not available)

RESULTS

The results of this study are summarized in Table 1 to which reference should be made for details pertaining to the following discussion. Representative types of responses are shown in figures 1 to 9, plates I and II. Study of these will show that there is a period of re-orientation of the spiral artery which reaches a maximum at seventy-two hours after injection of the gonadotrophin, or about sixty hours after ovulation. Following this, there is a restitution of the spiral configuration, although it is on a larger scale in an enlarged

ovary than at the onset of the induced pseudopregnancy. The factors concerned with the recovery of coiling in the spiral artery have not been ascertained.

The re-orientation of the spiral artery occurring within the first three days is made up of three elements. First, there is an increase in the distance between the coils of the spirals. Second, there is extension of the loops so that, by 48 to 72 hours after the injection, they present an undulating or sinuous appearance. Third, in some instances, but not all, lateral flattening of the coils takes place to a marked degree as the artery is extended longitudinally. The last characteristic may persist until after the ninth day (see figure 8, plate II). The result of these changes is that the spiral artery may actually lose its spiral characteristic either in part or *in toto* during the first three days following intense gonadotrophic stimulation as may be seen in figures 4 and 5. By the sixth to the ninth day, the artery largely recovers its spiral configuration with the exception of possible retention of the flattened characteristic (see figure 9b).

SUMMARY

The ovarian artery gives rise to one or two spiral arteries in the hilus of the ovary. Each forms a helix of diminishing diameter lying along the length of the ovary. The arterial supply to the stroma of the ovary is derived from the coils along these spiral vessels.

In isolated female rabbits receiving gonadotrophic substances intravenously, a pattern of changes, or re-orientation, takes place during the period of rapid ovarian enlargement associated with generalized growth of Graafian follicles, ovulation, and luteinization.

The features of adaptive alterations of the spiral vessels to increased size of the ovary consist of, (1) an increase in the distance between the coils of the spiral as this vessel is extended; (2) an extension of the loops so that by 48 to 72 hours after stimulation of the ovary with gonadotrophins the spiral arteries present a partially or completely undulating appearance; and (3) a lateral flattening of the coils of the spiral vessel.

Except for the flattening which has been seen in ovaries taken as late as the ninth day of pseudopregnancy, the spiral configuration is largely restored by the sixth day, even though the ovary is still quite large.

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DISTORTION OF THE SPIRAL ARTERY IN THE OVARY ASSOCIATED WITH CORPUS HEMORRHAGICUM CYSTS

S. R. M. REYNOLDS

From the Department of Embryology, Carnegie Institution of Washington
BALTIMORE 5, MARYLAND

THE SPIRAL artery in the hilus of the ovary permits adaptation of the main arterial supply of the ovary to an increase in size of the ovary following stimulation by gonadotrophins (Reynolds, 1947a). It has been suggested that another of the principal consequences of this spiral arrangement of the arterial supply of the ovary is to bring about a reduction and equalization of blood pressure throughout the stroma of the ovary (Reynolds, 1947a, b). With the foregoing considerations in mind it was surmised also that abnormal relations with respect to the foregoing mechanisms might play a role in the etiology of certain pathological conditions in the ovary whose cause is now obscure.

In the present communication, evidence is submitted to show that there is in fact an association between irregular adaptation of the spiral artery in response to ovarian enlargement and the presence of corpus hemorrhagicum cysts.

MATERIALS

A series of female rabbits received gonadotrophins for the purpose of studying adaptation of the spiral artery to ovulation and luteinization (Reynolds, 1947a). A certain number of ovarian responses were obtained in which there were multiple large Graafian follicles and one or more corpus hemorrhagicum cysts. The latter were two or more millimeters across or about twice the normal size of Graafian follicles in the rabbit ovary. Vinylite injection-corrosion casts of the larger ovarian blood vessels were obtained in the manner described before (Reynolds, 1947b). Photographs were made of the fresh ovaries, and as in the previous study, stereoscopic photographs of the casts were made by Mr. Chester F. Reather, photographer in this department. With these records available, it was possible to compare the location of various parts of the spiral artery with the location of cysts in the ovary.

RESULTS

The essential results of this study are shown in the figures of plates I and II, and are summarized briefly as follows:

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a. At *twenty-four hours* after injection of gonadotrophins, small corpora hemorrhagica have been seen in association with newly ovulated follicles and large, clear follicles. In two such instances, one of which is shown in figure 1, the coils of the spiral artery are "paid out" in response to the generalized follicular stimulation, but they were quite regularly formed. This extension of the spiral artery compares indistinguishably with that which occurs in connection with normal ovulation and follicle-growth.

b. At *forty-eight hours* after injection of gonadotrophins, one ovary was obtained in which three corpora lutea were forming and there was one very large corpus hemorrhagicum cyst. The coils of the spiral artery were evenly extended except at one point. Here, one coil was pulled out into a nearly straight position. By comparing the blood-vessel cast with a photograph of the fresh ovary it is possible to state that the cyst was immediately distal to the straightened coil (see figure 2). It should be noted that the caudal ends of the ovary in the two pictures are reversed. The contralateral ovary without a cyst yielded a spiral artery of well-formed but extended configuration.

c. At *seventy-two hours* after administering gonadotrophins an ovary was obtained which had two corpora lutea and three corpus hemorrhagicum cysts. In this case, there was marked irregularity of the spiral artery, with the coils much extended. Unfortunately, the veins had been injected so heavily with blue vinylite that exact details in the spiral artery could not be distinguished further.

d. At *ninety-six hours* after injecting gonadotrophins, three ovaries containing corpus hemorrhagicum cysts were obtained. In each, the cast of the spiral artery could be clearly seen and studied. In all three, there was marked disruption of the coiled arrangement, and the cysts were distal to the region of disruption. Examples of these are shown in figures 4 and 5. Attention is called to the latter, particularly. Here, the corpus hemorrhagicum cysts were limited to the cephalic end of the ovary, with normal corpora lutea at the caudal end. This is shown in figure 5a. Figure 5b shows the disruption of the major spiral artery, whereas figure 5c, taken at a higher magnification (×8) and from another angle, shows the regular, although extended coils of the secondary spiral artery in the caudal pole of the ovary despite the presence of large terminal arteries.

e. At *six days* after gonadotrophin administration, one pair of ovaries was obtained in which small corpora hemorrhagica, not cystic, were seen in association with new corpora lutea and large, clear follicles. The spiral arteries in these have been described as being extended, and flattened laterally (Reynolds, 1947a).

A striking contrast to this was seen in another pair of ovaries which contained many corpus hemorrhagicum cysts. No corpora lutea were grossly discernible in these ovaries. The uterus was hyperemic but infantile. The ovarian spiral arteries from one of the ovaries,

shown in figure 6b, were irregularly extended, particularly the one passing cephalad.

f. *Nine and twelve* days after injection of gonadotrophins, ovaries were obtained which had four to six recent corpora hemorrhagica. These were of small diameter (approximately one millimeter). In none of these ovaries was there any marked difference in the configuration of the spiral arteries from those obtained in ovaries in which corpora hemorrhagica were absent (cf. Reynolds, 1947a). Accordingly, if these spiral arteries presented at an earlier time the aspects of distortion described in *b*, *c*, and *d* above, it is clear that the spiral arteries have recovered to a large degree from extreme distortion. If follows, therefore, that spiral characteristics re-appear in the vessel concerned despite an over-all increase in size of the ovary at this time, compared to the size of the non-stimulated ovary, just as they do in ovaries in which only luteinization of ovulated follicles takes place.

COMMENT

The contrast between the regular configuration of the spiral artery during ovulation and luteinization and marked localized distortion of a part of the vessel central to the location of corpus hemorrhagicum cysts suggests that there is a causal relation between normal paying out of the spiral vessel and the normal response of ovulation with subsequent luteinization. Similarly, there appears to be causal relation between distortion of the artery and cyst-development after stimulation of the ovary by gonadotrophins. If, as has been suggested (Reynolds, 1947b), the function subserved by the spiral artery is that of lowering and equalizing blood pressure at near-osmotic levels in the ovary, it should follow that an irregular configuration during a phase

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FIGS. 1a–4b

FIG. 1a. Fresh[1] ovary, 24 hours after gonadotrophins. Four corpora lutea, several small corpora hemorrhagica. ×2. Caudal pole to left.

FIG. 1b. Spiral artery of ovary shown in 1a. Note extended coils midway along artery. ×3. Caudal pole at right.

FIG. 2a. Fresh ovary, 48 hours after gonadotrophins. Three corpora lutea, one large corpus hemorrhagicum cyst. ×2. Caudal pole to right.

FIG. 2b. Spiral artery (veins shown also) of same ovary shown in 2a. Note distorted coil midway along artery, with cyst lying distal to this. Caudal pole to left.

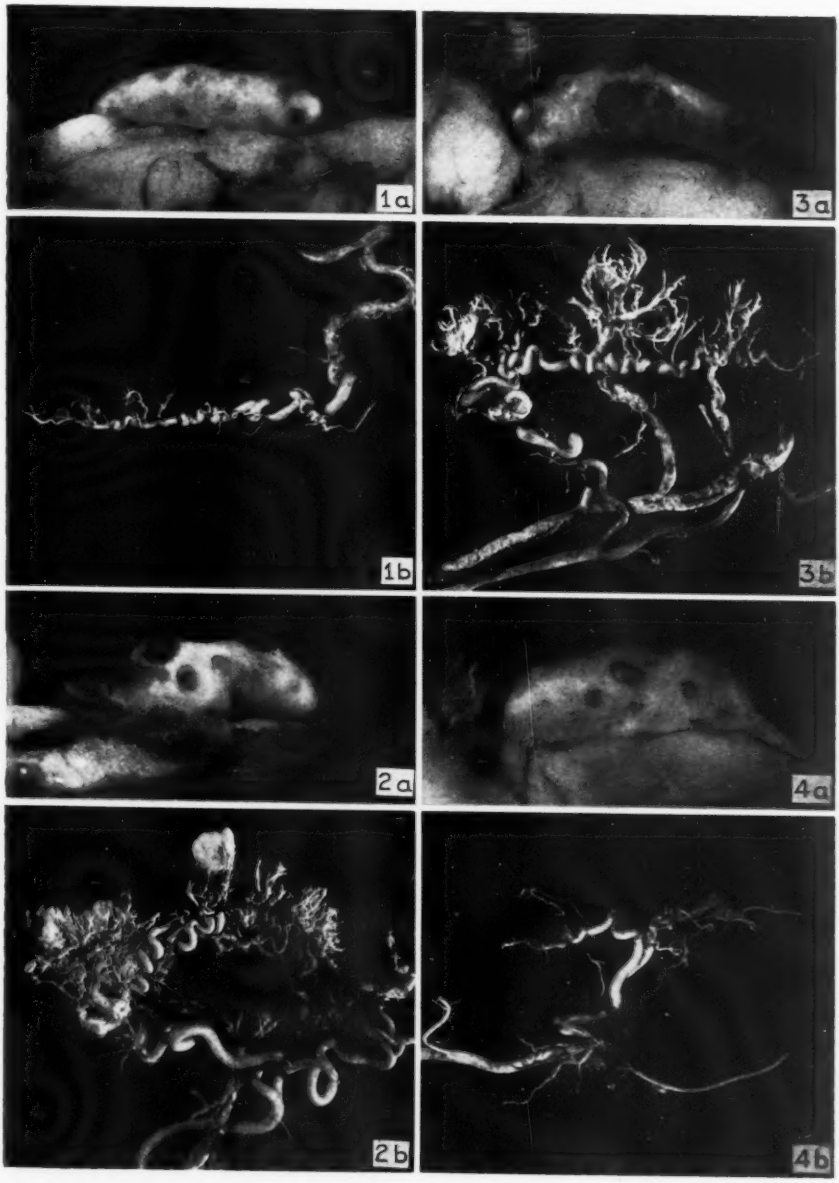
FIG. 3a. Fresh ovary, 48 hours after gonadotrophin. Six corpora lutea, 2 corpus hemorrhagicum cysts, many large follicles. ×2. Caudal pole to right.

FIG. 3b. Spiral artery (and veins) of ovary shown in 3a. Note extended coil central to location of two cysts. ×3. Caudal pole to left.

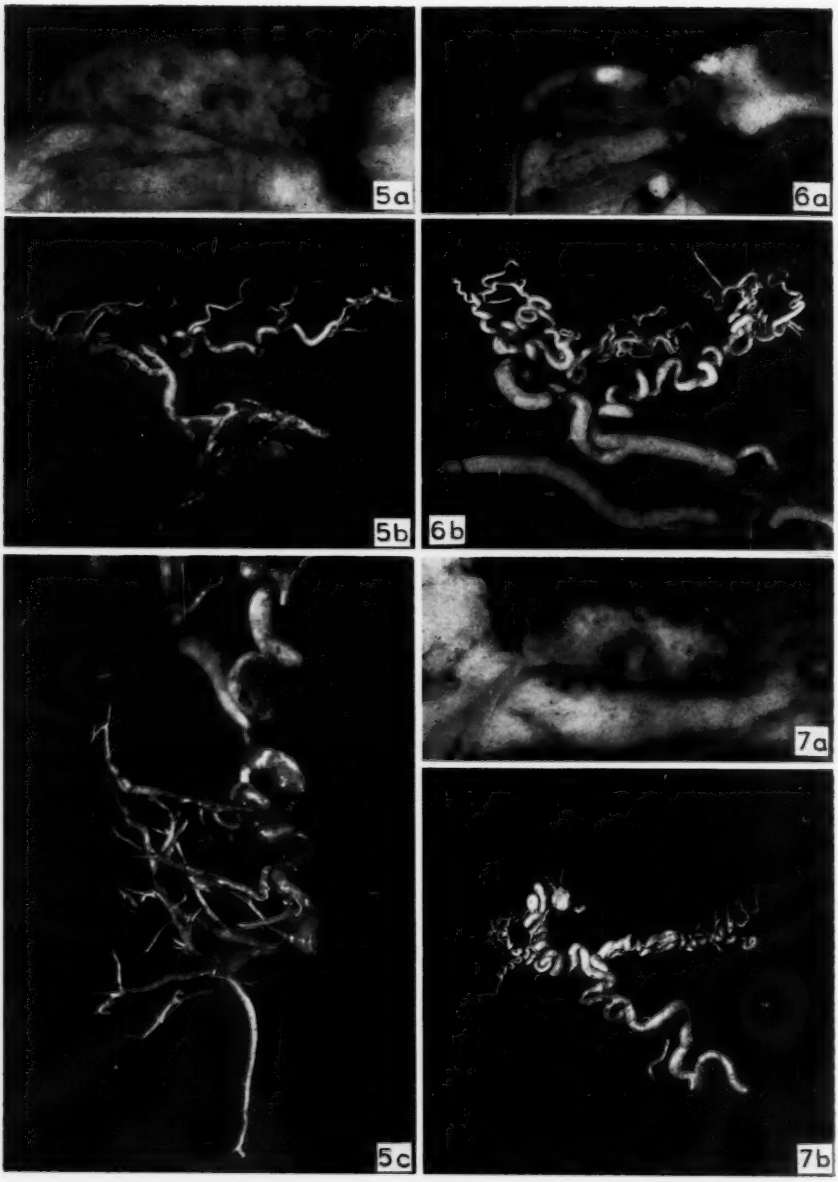
FIG. 4a. Fresh ovary, 96 hours after gonadotrophin. Several corpora lutea; several corpora hemorrhagica. ×2. Caudal pole at right.

FIG. 4b. Extended spiral arteries of ovary shown in 4a. Note straightened coils. ×3. Caudal pole at left.

[1] Fresh ovaries injected with vinyl acetate.



FIGS. 1a–4b



FIGS. 5a–7b

of rapid follicular development should allow an unequal distribution of blood pressure in the ovary. The principal hemodynamic conditions determining the quantity of kinetic energy transmitted by blood to the terminal arteries include the pressure in the spiral artery; the length of artery; the diameter of the vessel; a change in the direction of flow; the angle at which a terminal artery arises from the axis of the spiral vessel; and the ratio of the size of the branching vessel to the stem from which it originates (see references in Reynolds, 1947b).

A concept of the situation with respect to development of follicular cysts which accords with established physiological mechanisms is, therefore, that unequal growth of a Graafian follicle or a group of follicles disrupts the orderly arrangement of the coils of the artery. This, in turn, allows too great a pressure to be transmitted to the adjacent tissues. As a result, excessive passage of fluid takes place from the capillary bed supplied by branches from the distorted loop. The only structure which can accommodate excess fluid in the compact structures of the ovary appears to be the Graafian follicle. Further distortion of the spiral vessel should accompany enlargement of a cyst. According to this view, the development of corpus hemorrhagicum cysts is to be regarded as analogous to an edematous condition in other vascular beds in the body.

SUMMARY

Corpus hemorrhagicum cysts were induced in the ovaries of rabbits by injection of gonadotrophins. Injection-corrosion preparations of the vessels, using vinylite as the injection-mass, were studied by means of stereoscopic pictures.

In contrast to the normal pattern of adaptation of the spiral artery to ovarian growth in which there is relatively even paying out of the coils, distortion of localized portions of the artery was observed in the

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FIGS. 5a–7b

FIG. 5a. Fresh ovary, 96 hours after gonadotrophin. Several corpora lutea at one end, corpora lutea and corpus hemorrhagicum cysts at other. ×2. Caudal pole at left.

FIG. 5b. Spiral arteries of ovary shown in 5a. Note extended spiral artery on right. See Fig. 5c for details of other spiral. ×3. Caudal pole on left.

FIG. 5c. Spiral artery, caudal branch, shown in 5b, above. Note regular configuration, despite large terminal arteries which supplied several corpora lutea (see 5a). ×8.

FIG. 6a. Fresh ovary 6 days after gonadotrophin. Corpora hemorrhagica only; no corpora lutea. Uterus was infantile. ×2. Caudal pole at left.

FIG. 6b. Twisted spiral arteries of ovary shown in 6a. Note extended coils, especially in spiral artery on the right. ×5. Caudal pole at the left.

FIG. 7a. Fresh ovary, 9 days after gonadotrophin. Four corpora lutea; four corpora hemorrhagica, numerous medium and large sized follicles. ×2. Caudal pole at left.

FIG. 7b. Spiral arteries of ovary shown in 7a. Tightly twisted coils are found in an enlarged ovary. Note somewhat extended coil in mid-point of right spiral artery, directly under a cyst (see 7a). ×3. Caudal end at left.

vicinity of corpus hemorrhagicum cysts. The cysts were distal to the distorted part of the spiral artery.

It is suggested that distortion of the spiral artery may contribute to development of the cysts through the mechanism of transmission of too great a local pressure of blood during the growth phase of the follicle under stimulation of gonadotrophins.

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THE EFFECTS OF HYPOPHYSECTOMY ON GASTRIC ACIDITY OF ADULT FEMALE RATS[1]

ROGER C. CRAFTS AND BURNHAM S. WALKER[2]
From the Departments of Anatomy and Biochemistry,
Boston University School of Medicine
BOSTON, MASSACHUSETTS

HYPOPHYSECTOMY induces an anemia in adult male or female rats. This anemia is microcytic and hypochromic, accompanied by hypoplasia of the bone marrow (Crafts, 1946). Injections of a combination of thyroxine, iron, and copper alleviated the anemia (Crafts, 1946). This suggests that the metabolism of iron may be disturbed following hypophysectomy. All phases of iron metabolism are being studied in normal and hypophysectomized female rats.

Normal gastric acidity is generally recognized as a significant factor in iron absorption. This paper is concerned with changes in the acidity of gastric secretion following removal of the hypophysis.

METHODS

Adult female rats of the Long-Evans strain and of 3 to 4 months of age were used. They were maintained on Rockland Farms complete rat diet and fresh lettuce once a week.

Hypophysectomy was performed by the parapharyngeal approach under ether anesthesia.

Gastric samples were obtained in the following manner: after fasting for 24 hours, each rat was anesthetized with ether and 0.1 cc. of blood was removed via heart puncture for erythrocyte and hemoglobin determinations. Ether anesthesia has no effects on the erythrocyte count or hemoglobin level of heart blood (Crafts, 1944). The abdomen was then opened, a tie placed around the gastroduodenal juncture, and the stomach contents removed via syringe and 18 gauge needle (gastric sample #1). One half hour after the first sample was removed, gastric sample #2 was removed. Each animal was then given a subcutaneous injection of mecholyl. The dose was 0.04 mg. per kilo of body weight. Gastric samples #3 and #4 were obtained one half hour and one hour after the mecholyl injection.

Erythrocyte counts were made with U. S. certified pipettes and with the improved Neubauer counting chamber. The hemoglobin was determined with an Evelyn photoelectric colorimeter.

Free acid, combined acid, and pH determinations were made on each gastric sample. The volume of each sample was measured in a tuberculin syringe. The sample was then diluted to a uniform volume of 5.0 cc. with

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carbon dioxide free water, and the pH determined using a glass electrode. One half cc. samples of the diluted gastric samples were titrated using phenolphthalein as the indicator for total acidity, and Toepfer's reagent for free hydrochloric acid. Titrations were carried out with a 0.1 N potassium hydroxide solution, in a Rehburg microburette.

RESULTS

The rats were divided into three groups, (1) normal control rats, (2) hypophysectomized rats, and (3) rats bled from the heart over a two week period. The last group was studied as a further control because of the possibility of decreased gastric acidity resulting from anemia. Table 1 shows the figures obtained on the erythrocyte count and hemoglobin level. The erythrocyte count for the normal control rats was 8.40 (S.D. 0.70)[3] million cells per cu. mm. The hemoglobin level was 15.1 (S.D. 0.63) gm. per 100 cc. Hypophysectomy induced

TABLE 1. AVERAGE ERYTHROCYTE AND HEMOGLOBIN VALUES FOR CONTROL, HYPOPHYSECTOMIZED, AND BLED RATS

	Control rats (11)	Hypophysectomized rats (12)	Bled rats (10)
Erythrocyte count in millions per cu. mm.	8.40 ±0.70	5.60 ±0.47	5.42 ±0.69
Hemoglobin in gm. per 100 cc.	15.1 ±0.6	9.3 ±0.6	9.1 ±1.5

± = Standard Deviation.

the usual anemia (Table 1). The erythrocyte count decreased to 5.60 (S.D. 0.47) million cells per cu. mm., and the hemoglobin decreased to 9.3 (S.D. 0.60) gm. per 100 cc. Blood was removed from the bled rats until they exhibited erythrocyte and hemoglobin levels similar to the hypophysectomized rats, i.e. 5.42 (S.D. 0.69) million cells per cu. mm. and 9.1 (S.D. 1.5) gm. per 100 cc.

After anemia had developed in the hypophysectomized and bled rats, each rat was anesthetized and gastric samples obtained and studied by the methods already described.

Gastric Sample #1.—Table 2 shows the results obtained from gastric sample #1, taken immediately after ether anesthesia. This would represent the gastric acidity in the animal under usual circumstances. The total acid per sample for the control rats averaged .0216 m. eq.;[4] for the hypophysectomized rats, .0094 m. eq.; a difference of .0122 m. eq. The average volume of the samples from the control rats was 0.38 cc., and the hypophysectomized rats, 0.23 cc. Calculating the amount of acid per liter it was found that the normal controls showed 59.8 m. eq. per liter, and the hypophysectomized rats 46.4 m. eq. per liter.

[3] Standard deviation.
[4] Milli equivalents.

A glance at Table 2 will show that the difference in total acid per sample and in volume are significant but that the difference in total acid per liter, with the large standard deviations, is not significant. These data would indicate that the hypophysectomized animals produced gastric juice of normal acidity but of decreased volume. The average of the pH values obtained for the normal control rats was 3.30; for the hypophysectomized rats, 4.40.

A decreased gastric acidity did not accompany the anemia produced by bleeding from the heart. The total acid per sample averaged .0221 m. eq. for the bled rats. The difference between the bled rats and hypophysectomized rats was .0127 m. eq. per sample. The difference in volume was 0.07 cc., which was not a significant difference. Calculating the total acid per liter, it was found that the hypophysectomized rats had 46.4 m. eq. per liter; the bled rats, 78.5 m. eq.; a difference of 32.1 m. eq. Using the same reasoning that was used in comparing the hypophysectomized and control rats, we found that the anemic rats did not produce a decreased gastric acidity and, as the difference in volume produced by the hypophysectomized and bled rats was not significant, that normal rats under anemic conditions were able to produce a more concentrated gastric juice than that produced by hypophysectomized rats. The average of the pH values obtained for the bled rats was 3.69 compared to 4.40 for the hypophysectomized rats.

Figures for free acid showed that the hypophysectomized rats had less free acid (.0001 m. eq. per sample) than either the normal controls (.0037 m. eq. per sample) or the bled rats (.0048 m. eq. per sample). Of more significance, however, is the finding that 6 of 11 normal control rats (54%), and 6 of 10 of the bled rats (60%) had free acid in gastric sample #1, while only 1 of 12 hypophysectomized rats (8%) showed the presence of free acid.

In summary, the gastric juice of hypophysectomized rats obtained immediately after anesthesia showed a decrease in volume, a decreased incidence of free acid, and an increase in pH values. The acidity of the gastric secretion was not significantly reduced. Normal rats made anemic did not exhibit these decreases. The figures indicated that the gastric juice of bled rats had a higher acidity than that of the hypophysectomized rats.

Gastric Sample #2—This sample was taken one half hour after gastric sample #1 and is an indication of how much and the type of gastric juice each rat can produce during 30 minutes of ether anesthesia.

Table 2 shows the figures obtained. Although the average figures indicated a decreased acid production by the hypophysectomized animals, the statistics of the difference between the means showed that these averages were lacking in significance.

When the two groups of anemic animals were compared, one with the hypophysis intact and the other with this gland removed, a

TABLE 2. THE EFFECTS OF HYPOPHYSECTOMY ON GASTRIC ACIDITY OF ADULT FEMALE RATS

	Gastric sample #1			Gastric sample #2			Gastric sample #3			Gastric sample #4		
	Con.	Hyp	Bled	Con.	Hyp.	Bled	Con.	Hyp.	Bled	Con.	Hyp.	Bled
Number of rats	11	12	10	11	12	8	10	12	7	10	9	6
Volume of each sample in cc.	0.38 ± .12	0.23 ± .09	0.30 ± .12	0.30 ± .07	0.34 ± .13	0.43 ± .10	0.37 ± .10	0.23 ± .12	0.36 ± .18	0.28 ± .08	0.17 ± .06	0.31 ± .05
	t=3.1	t=1.3		t=0.9	t=1.6		t=2.8	t=1.8		t=3.1	t=4.0	
pH of each sample	3.30 ± .24	4.40 ±1.03	3.69 ± .29	4.35 ± .50	5.20 ±1.46	3.93 ± .33	4.11 ± .39	5.54 ±1.30	3.58 ± .28	4.34 ± .58	6.30 ± .75	3.67 ± .23
	t=3.3	t=2.0		t=1.7	t=2.2		t=3.2	t=3.7		t=5.6	t=7.7	
Total acid per sample—milli equiv.	.0216 ± .0068	.0094 ± .0038	.0221 ± .0081	.0146 ± .0042	.0112 ± .0054	.0286 ± .0091	.0207 ± .0054	.0093 ± .0034	.0360 ± .0157	.0149 ± .0041	.0077 ± .0024	.0227 ± .0086
	t=5.2	t=4.6		t=1.5	t=5.5		t=5.8	t=12.2		t=4.2	t=4.8	
Total aid per liter—milli equiv.	59.8 ±17.8	46.4 ±20.4	78.5 ±21.9	49.3 ± 8.9	41.4 ±17.0	65.2 ±17.9	56.8 ±10.8	45.0 ±22.3	104.8 ±23.1	56.7 ±21.5	49.2 ±24.2	70.1 ±18.0
	t=1.6	t=3.8		t=1.5	t=3.3		t=1.5	t=5.9		t=0.5	t=1.7	
Free acid per sample—milli equiv.	.0037	.0001	.0048	0	0	.0027	.0011	.0003	.0093	0	0	.0014
Free acid per liter—milli equiv.	21.2	0.67	13.6	0	0	0.89	2.54	1.78	20.7	0	0	3.6

± =Standard Deviation
t=Fisher's t values; if 2.0 or greater, data are significant; if less than 2.0, data are not significant.

Presence of Free Acid

G_1— 6	control rats; 1	hypophysectomised rat; 6	bled rats.
G_2— 0	control rat; 0	hypophysectomised rat; 2	bled rats.
G_3— 2	control rats; 1	hypophysectomised rat; 5	bled rats.
G_4— 0	control rat; 0	hypophysectomised rat; 1	bled rat.
8	2	14	
19.0%	4.4%	45.1%	

significant difference was found. The average total acid per sample value for the hypophysectomized rats was .0112 m. eq.; for the bled rats, .0286 m. eq.; a difference of .0174 m. eq. per sample. The total acid per liter for the hypophysectomized rats was 41.4 m. eq.; the bled rats 65.2 m. eq.; a difference of 23.8 m. eq. per liter. The difference in volume of gastric juice obtained was not significant. The average of the pH values obtained was 5.20 for the hypophysectomized rats and 3.93 for the bled rats. Thus, rats made anemic by bleeding from the heart produced a gastric secretion of higher acidity than that produced by hypophysectomized rats.

Free acid was found in 25% of the bled rats but in no control or hypophysectomized rats.

Summarizing, no significant difference was found between the normal control and the hypophysectomized rats in the amount or acidity of gastric juice produced in a 30 minute period of ether anesthesia. The anemic rats with an intact hypophysis, however, produced gastric secretion of a higher acidity and lower pH than found in hypophysectomized rats.

Gastric Sample #3—These samples were obtained 30 minutes after a subcutaneous injection of mecholyl, a known gastric stimulant (0.04 mg. per kilo of body weight).

Table 2 shows the results obtained. A significant difference between normal control rats and hypophysectomized rats was found in total acid per sample (Con. .0207—Hyp. .0093 m. eq.), in volume (Con. 0.37—Hyp. 0.23 cc.), in pH values (Con. 4.11—Hyp. 5.54), and in free acidity. The difference between the total acid per liter (Con. 56.8—Hyp. 45.0 m. eq.) was not significant. The hypophysectomized animals, therefore, produced gastric secretion of normal acidity but of decreased volume.

A significant difference between hypophysectomized rats and the bled rats was found in total acid per sample (Hyp. .0093—Bled .0360 m. eq.), in total acid per liter (Hyp. 45.0—Bled 104.8 m. eq.), and in pH (Hyp. 5.54—Bled 3.58). The difference in volume (Hyp. 0.23—Bled 0.36 cc.) was not significant. The bled animals produced gastric secretion of higher acidity than that produced by hypophysectomized rats.

Five of 7 of the bled rats (71%) showed the presence of free acid while only 2 of 10 of the control rats (20%) and 1 of 12 of the hypophysectomized rats (8%) had free acid in the stomach.

In summary, normal control rats responded to mecholyl by producing a greater volume of gastric juice than the hypophysectomized animals, and the bled rats produced gastric secretion of higher acidity than that produced by hypophysectomized rats. The pH values were higher in the hypophysectomized rats than in either the normals or the bled rats. These results are the same as found in animals immediately after ether anesthesia (gastric sample #1).

Gastric Sample #4—These samples represent the acidity of the gastric juice produced in a 30 minute period, starting 30 minutes after the mecholyl injection and ending 60 minutes after said injection.

The normal control animals produced .0149 m. eq. of acid per sample; the hypophysectomized animals, .0077 m. eq.; a difference of .0072 m. eq. A significant difference was also found in volume (Con. 0.28—Hyp. 0.17 cc.), and in the pH (Con. 4.34—Hyp. 6.30). The difference between the total acid per liter in the two groups (Con. 56.7—Hyp. 49.2 m. eq.) was not a significant difference. These figures again indicated that the hypophysectomized rats produced a decreased volume of gastric secretion of normal acidity.

Comparing the bled rats with the hypophysectomized animals, it was found that the difference in the total acid per sample (Hyp. .0077 —Bled .0227 m. eq.), the volume (Hyp. 0.17—Bled 0.31 cc.), and the pH (Hyp. 6.30—Bled 3.67) were significant differences. The difference in total acid per liter between the hypophysectomized rats and bled rats (Hyp. 49.2—Bled 70.1 m. eq.), although large, is not significant. Thus, contrary to the other three gastric samples, the bled rats produced gastric secretion of greater volume than found in hypophysectomized animals but of normal acidity rather than of increased acidity.

No control rat or hypophysectomized rat produced free acid, and only 1 of 6 of the bled rats had free acid.

Summarizing, hypophysectomized rats, in a 30 minute period starting one half hour after an injection of mecholyl, produced less gastric secretion than was produced by normal rats or rats made anemic by bleeding from the heart, and produced gastric juice of a higher pH value.

Hypophysectomized rats would seem to be unable to produce gastric secretion of equal volume to that produced by normal rats of the same age, but the acidity of that produced is equal to that of control rats. Rats made anemic by bleeding from the heart produced gastric secretion of higher acidity than that produced by hypophysectomized animals (except in gastric sample #4 where there was an increased volume only). Free acid was found in 19% of all gastric samples taken from normal control rats, in 4% of samples from hypophysectomized rats, and 45% of all samples taken from the bled rats.

DISCUSSION

Hypophysectomy induced a decrease in the volume of gastric secretion in the adult female rat. Although hypophysectomy may induce this decrease in gastric secretion by altering factors which have nothing to do with the endocrine system, the effects of other endocrine glands and products on gastric secretion should be mentioned.

The posterior lobe of the pituitary has been found to have an influence on gastric secretion. Metz and Lackey (1940) removed the posterior lobe in dogs and obtained an increase in acidity and volume

of gastric secretion. Blotner (1940) found similar results in patients with diabetes insipidus. DeAncias (1926) reported that injections of pituitrin caused a decrease in acidity and volume of gastric secretion in man, while Cutting, Dodds, Noble, and Williams (1937) found, in cats, that pituitrin injections decreased the volume of gastric secretion but did not alter the acidity. Contrary to the above results, Cutting, Dodds, Noble, and Williams (1937), in another paper, found a decreased gastric acidity following hypophysectomy in cats but the volume-acidity ratio was upset. These workers found an increased volume of secretion in some hypophysectomized animals and a decrease in others. There was always a decreased total acidity, however. These authors found that the removal of the anterior lobe alone did not produce these changes, and thus claim that loss of the posterior lobe is responsible for the decreased acidity in hypophysectomized animals. Gross, Ingram, and Fugo (1942) reported decreased volume with no change in acidity in dogs when the pituitary stalk was cut. These workers also found that pituitrin injections returned the volume of gastric secretion to normal without altering the acidity. These results are similar to ours for hypophysectomized rats.

The data on the effects of the thyroid gland on gastric acidity are very confusing. Lerman and Means (1932) reviewed the contradictory literature and reported that patients with exophthalmic goiter and those with myxedema both exhibited a decreased gastric acidity. When corrected for age distribution, these authors found that the incidence of decreased gastric acidity in hyperthyroidism was 46%; in hypothyroidism, 75%.

Injection of extracts of pregnancy urine have been found to decrease gastric acidity in dogs (Culmer, Atkinson, and Ivy, 1939), and Sandweiss, Saltstein, and Farbman (1938) found that pregnancy urine extracts were potent in preventing the formation of jejunal ulcers in dogs. In addition, Way (1945) found that gastric acidity varied inversely with the amount of anterior pituitary-like factor found in the urine of the human.

Babkin, Komarov, and Komarov (1939) reported that injections of parathormone would decrease both volume and acidity of gastric secretion in dogs. Shiffrin (1942) confirmed these results and found, in addition, that thyroparathyroidectomy increased gastric acidity.

Estrogens were found to have an inhibitory influence on the production of gastric secretion (Winkelstein, 1936). Atkinson and Ivy (1938), in dogs and humans, and Schiff, Felson, Graff, and Meyer (1938), in the human, found that injections of estrogens had no influence on gastric acidity.

Tuerkischer and Wertheimer (1945) have reported a marked decrease in gastric secretion following adrenalectomy in rats.

Of the above findings, the decreased activity of the adrenal cortex and the loss of the posterior lobe as a result of hypophysectomy may be causes for the decrease in gastric secretion in the hypophysec-

tomized rat. The actual explanation of the decreased gastric secretion following hypophysectomy will have to await further work.

Our results on gastric acidity in normal rats agree with Komarov, Shay, Rayport, and Fels (1944) and Friedman (1943) that there is a spontaneous gastric secretion in the rat; and disagree with Machella and Griffith (1942) who reported finding no free acid in the gastric juice of rats.

Hypophysectomy induced a decrease in gastric secretion in adult female rats. That this decrease is sufficient to account for the profound anemia which is induced by hypophysectomy is doubtful. It may, however, be a factor in altering the metabolism of iron in the rat following hypophysectomy.

SUMMARY

Adult female rats were found to have a spontaneous secretion of gastric juice, which frequently contained free hydrochloric acid.

Hypophysectomy induced a decrease in the volume of gastric secretion and an increase in pH values.

Rats made anemic by bleeding from the heart produced gastric juice which was of higher titratable acidity and lower pH than that produced by hypophysectomized rats.

CONCLUSION

Hypophysectomy induced a decrease in volume of gastric secretion which was not due to the anemia which followed hypophysectomy in the adult female rat.

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THE IODIDE CONCENTRATING MECHANISM OF THE RAT THYROID AND ITS INHIBITION BY THIOCYANATE[1]

J. E. VANDERLAAN AND W. P. VANDERLAAN[2]
From the Department of Medicine, Tufts College Medical School and the Joseph H. Pratt Diagnostic Hospital
BOSTON, MASS.

IT IS NOW generally agreed that antithyroid drugs such as thiouracil prevent the formation of thyroid hormone. Astwood and Bissell (1944) found in rats that under the continuous influence of thiouracil the iodine content of the thyroid rapidly falls to low levels and that the thyroid gland simultaneously enlarges. Astwood (1944–45)showed that animals thus depleted of iodine are still able to concentrate rapidly considerable quantities of iodine when injected with potassium iodide. This iodine is not precipitated when the thyroid protein is denatured by heating. McGinty (1946) has noted that increasing the amount of dietary iodine raises the thyroid iodine content above the usual low levels in rats chronically treated with thiouracil. This increased thyroid iodine is soluble in the supernatant fluid when the protein of the gland is precipitated with Somogyi's reagent. VanderLaan and Bissell (1946) found that iodine accumulated in the thyroid after injection into the animal depleted with thiouracil is retained by the thyroid for only a short period. It would seem unlikely that thyroid hormone would disappear so rapidly. Also since the iodine of thyroid hormone is intimately bound to protein, it should be precipitated with the protein when the latter is denatured. Since this does not occur it seems that appreciable concentrations of iodine (which clearly are not thyroid hormone) can exist in the thyroid. Using radioactive iodine as an indicator to measure the incorporation of iodide into thyroxine and diiodotyrosine by excised thyroid tissue, Schachner, Franklin and Chaikoff (1944) found an inorganic fraction after potassium hydroxide hydrolysis of thyroid slices previously incubated with sulfanilamide and azide. This fraction was assumed to represent iodide. Direct evidence that the iodine which is initially concentrated in the thyroid gland is iodide will be presented in this paper. In addition some of the relations between serum iodide and thyroid iodide levels in normal and hyperplastic glands will be reported.

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[2] Fellow the American Cancer Society.

Barker et al. (1936–41) in treating 246 cases of hypertension with thiocyanate reported 11 cases of goiter which were usually associated with clinical features of hypothyroidism. Prompt recovery occurred by giving 1 to 2 grains of thyroid daily. Astwood (1943) produced goiter in rats with thiocyanate, but found no goitrogenic effect, even with prolonged treatment, when iodide was given simultaneously. Since goitrogenic drugs of the thiourea and aniline series were not similarly modified by iodide administration it was concluded that a different type of action was involved. This conclusion has been supported by the work of Franklin, Chaikoff and Lerner (1944). They have studied the *in vitro* accumulation of radioactive iodide and its incorporation into diiodotyrosine and thyroxine by thyroid slices under the influence of various goitrogenic agents. Thiouracil and thiourea permitted iodide to be concentrated by thyroid slices but prevented its conversion into diiodotyrosine or thyroxine. Thiocyanate in similar concentration reduced the accumulation of iodine in addition. More recently further evidences of an effect of thiocyanate in impairing the ability of thyroid tissue to concentrate iodine has been reported. Wolff, Chaikoff, Taurog and Rubin (1946) found that thiocyanate depressed the iodine-concentrating capacity of the intact normal thyroid glands from rats maintained on low iodine diets provided the thiocyanate was administered shortly before the test was made. VanderLaan and Bissell used animals with thyroids depleted of iodine by the chronic administration of propylthiouracil. A moderate impairment of the usual ability of such thyroids to concentrate injected iodide occurred when these animals were fed thiocyanate in addition to propylthiouracil. If thiocyanate was injected into the animal shortly before the iodide was given almost complete inhibition of the usual iodine concentrating power occurred. Further studies will be presented here showing that thiocyanate also destroys the ability of thyroid cells to retain iodide which has previously been concentrated.

EXPERIMENTAL

Young rats of both sexes were fed a ground Purina mink chow diet containing 0.1% propylthiouracil, a concentration considerably in excess of that necessary to induce maximal thyroid hypertrophy. This was fed for two weeks or more, a period sufficient to deplete completely the gland of all stored hormonal iodine. Since thyroid glands from rats thus treated are able to concentrate large quantities of injected iodine, they are suitable for the study of the state of iodine as it is initially held by the thyroid. Into such animals doses of 200 micrograms of potassium iodide were injected subcutaneously, and 15 minutes later the animals were killed with chloroform, the thyroid glands were dissected and weighed, and they were then homogenized in distilled water in an amount such that each ml. of homogenate represented 50 mg. of thyroid tissue. This was ultrafiltered under pressure through a cellophane membrane. The nature of the iodine in the ultrafiltrate was studied by means both of an Heyrovsky Polarograph, using a dropping

mercury electrode, and potentiometrically with a silver-silver iodide electrode. In the potentiometric study the method as used by Hastings and VanDyke (1931) for bromide determination was adapted to the determination of micro quantities of iodide. 0.005N $AgNO_3$ was used and stirring was effected with a fine stream of nitrogen. Readings were obtained using the Cambridge pH meter as a potentiometer against a normal calomel electrode. One ml. of ultrafiltrate sufficed to determine with $\pm 5\%$ error an amount of iodine not exceeding 10 micrograms per ml. A horizontal microburette was used for the titration. In the chemical determination of the iodine content of thyroid tissue, the modification by Astwood and Bissell (1944) of Kendall's method was used to determine the total iodine. This was used to check the amount of iodide ion found by potentiometric titration.

In the study on the effects of thiocyanate the chemical determination of iodine in some instances was checked by labelling the injected iodine with radioactive iodine (I^{131}), removing an aliquot from the digestion mixture, evaporating it to dryness on small copper discs, and determining the amount of radioactivity, as compared to a standard treated in the same way. Potassium thiocyanate was usually injected intraperitoneally and potassium iodide in the amount of 100 micrograms, subcutaneously.

In studying the relationship existing between thyroid and serum iodide levels various doses of potassium iodide, labelled in each case with an identical amount of radioactive iodine (I^{131}) as tracer, were injected into rats. The rats were prepared in several different ways. The first group consisted of normal rats which received a tracer dose of radioactive iodide only. The second group of rats were given five milligrams of propylthiouracil intraperitoneally one hour prior to the injection of iodide. The first four rats of the second group of animals received tracer iodide only, the second four rats, tracer with one hundred micrograms of potassium iodide. The third group of animals was treated with propylthiouracil for sufficient period to deplete completely their hormone stores and were injected with labeled iodide in several dosage levels while still under the influence of the propylthiouracil. Four similarly treated rats were used to test each dose. A tracer dose alone and identical tracer doses added to 3, 10, 30, 100, 300, 1000, and 10,000 micrograms of potassium iodide were injected subcutaneously in 0.5 ml. of water. In all groups the animals were anesthetized with ether one hour after the iodide injection and blood drawn from the inferior vena cava. The animals were then killed with chloroform and the thyroids dissected out, weighed, and placed in exactly one ml. of 20% KOH. Digestion was accomplished by gentle heating. Evaporation was prevented by tightly stoppering the test tubes holding the samples. The rats in this experiment averaged 50 Gm. Each rat received approximately 10 microcuries of radioactive iodide. Counts with the Geiger-Müller counter were obtained as follows. Filter paper discs of equal size and quality were pasted with rubber cement onto copper discs 2.5 cm. in diameter. One tenth ml. of serum or digested thyroid was placed on the filter paper. The fluid spread itself uniformly through the paper which was then dried. Duplicates were run in all cases; these checked closely with the original determinations. The percentage of the total injected dose was determined by comparing with standards from the same sample of radioactive material added to normal serum and to thyroid tissue respectively, and prepared in similar fashion. The ratio of radioactivity between thyroid and serum was then determined.

RESULTS

The initial physical-chemical state of iodine concentrated by the thyroid gland: It has again been shown that under the influence of propylthiouracil, concomitant with the enlargement of the thyroid gland, the iodine of the thyroid is almost completely discharged and that such glands are able to concentrate iodine even though unable to

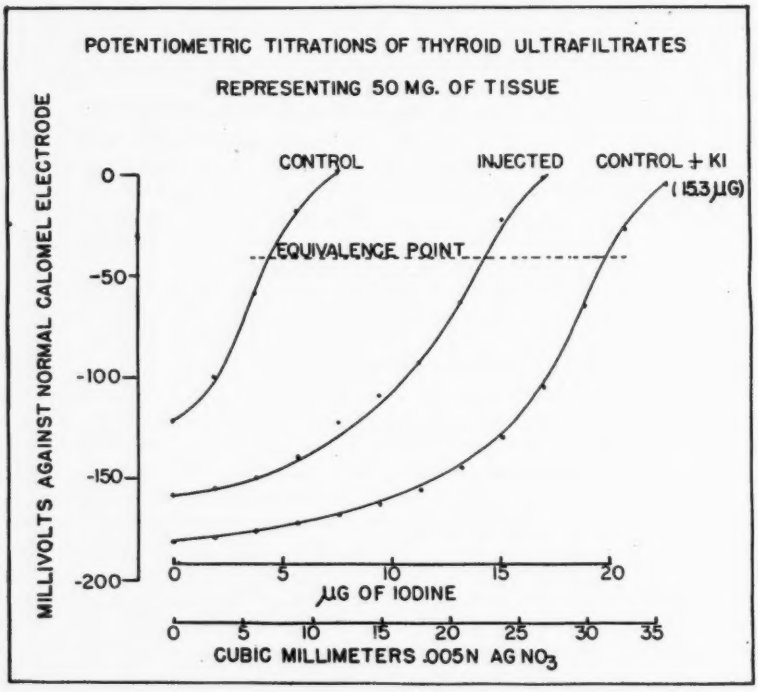

FIG. 1 shows the results of potentiometric titrations of ultrafiltrates of thyroid tissues removed from animals chronically treated with propylthiouracil. The control animals received no potassium iodide. The injected animals were given 200 micrograms of potassium iodide subcutaneously. In the third curve 15.3 micrograms of iodide were added to an aliquot of control ultrafiltrate.

incorporate it into thyroid hormone. When glands containing such high concentrations of iodine are homogenized in distilled water and ultrafiltered, iodine appears quantitatively in the ultrafiltrate. This was shown by chemical determinations and indicates that the iodine is freed from the thyroid with mechanical disruption of the cells. The iodine in the ultrafiltrate was studied both by polarographic and potentiometric methods. Although a wave characteristic of iodide ion could be obtained polarographically, constant and quantitative results were not obtained by this method. However, with the silver-

TABLE 1. QUANTITY OF IODINE FOUND IN 50 MILLIGRAMS OF THYROID TISSUE HOMOGENATE AND ULTRAFILTRATE AS DETERMINED BY CHEMICAL AND POTENTIOMETRIC ANALYSIS

	Control ultrafiltrate	Injected homogenate	Injected ultrafiltrate	Control ultrafiltrate +KI
Chemical	3.2 μg.	13.1 μg.	13.2 μg.	18.5 μg.
Potentiometric	4.2 μg.		14.3 μg.	19.9 μg.

Equivalence potential against normal calomel electrode taken to be −40 millivolts. 15.3 μg. iodide added to control ultrafiltrate (20 μg. KI).

silver iodide electrode reproducible results could be obtained and the iodine of the ultrafiltrate could be quantitatively accounted for as iodide ion. A typical graph of such a titration is shown in figure 1. Table 1 shows the results of this titration as compared to results obtained by chemical analysis. It can be noted that the addition of a known amount of KI to the ultrafiltrate of uninjected (control) thyroids results in a titration curve identical with the curve obtained by the ultrafiltrate of thyroid from iodide-injected animals. It is further to be noted that this added amount can be quantitatively measured by the method. This was repeatedly demonstrated. Since no chemical manipulations were involved it is apparent that iodide can be concentrated in considerable amounts in thyroid tissue under these conditions and that no bound iodine is present.

After it became clear that all the iodine concentrated in the thyroid under the conditions of our experiments is iodide, a study to determine the relationships existing between serum and thyroid iodide concentrations was made. The percentage of the total radioactivity exhibited by 0.1 ml. of serum was compared to a similar figure calculated from the radioactivity of 0.1 Gm. of thyroid tissue without correcting for specific gravity of serum. Results are shown in table 2 and graphically in figure 2. In animals treated acutely with propylthiouracil the thyroid concentrations of iodide as measured by radio-

TABLE 2. RATIO OF THYROID IODIDE TO SERUM IODIDE IN RATS INJECTED WITH INCREASING DOSES OF POTASSIUM IODIDE LABELLED WITH EQUAL TRACER DOSES OF RADIOACTIVE IODIDE

Hyperplastic thyroids								Anatomically normal thyroids	
Tracer only	3 μg. KI	10 μg. KI	30 μg. KI	100 μg. KI	300 μg. KI	1000 μg. KI	10,000 μg. KI	Tracer only	100 μg. KI
293	380	241	224	178	265			7.7	29.0
306	136	379	291	272	217			8.8	31.7
92	264	161	246	179	33	13	3.25	22.7	24.3
321	344	181	204	298	64	25	3.26	50.0	30.4
Av. 253	281	240	241	232	145	19	3.25	22.3	28.8

activity determinations averaged 25 times that of similar amounts of serum. This represents approximately the gradient maintained by normal thyroid tissue under these conditions. It was noted that a similar ratio between thyroid and serum iodide resulted both with tracer amounts of radioactive iodide and with tracer amounts plus 100 micrograms of potassium iodide as carrier. When the iodide con-

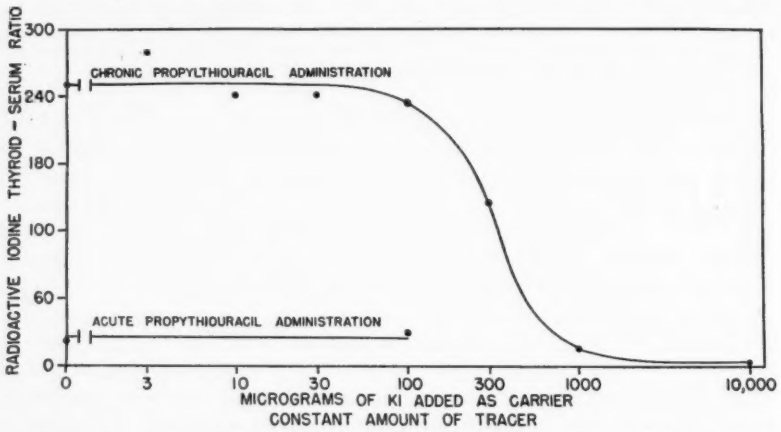

FIG. 2 shows that following the chronic administration of propylthiouracil the ratio of thyroid iodide to serum iodide is 250 to 1 over a wide dosage range. After the acute administration of propylthiouracil the ratio is 25 to 1 over the same range of iodide dosage.

centration of hyperplastic thyroid tissue from animals chronically treated with propylthiouracil was compared to the iodide concentration in the blood, the thyroid tissue iodide averaged 250 times that of serum over the entire range of carrier from 1 to 100 micrograms of potassium iodide. With higher doses of carrier the ratio between thyroid iodide and serum iodide became smaller, strikingly so with large doses. However, the thyroid iodide concentrating mechanism did not readily become saturated over the range tested as in each case increasing the amount of injected iodide caused a further increase

TABLE 3. THE EFFECT OF A SINGLE INJECTION OF PROPYLTHIOURACIL ON THE TOTAL UPTAKE OF IODINE IN THYROID TISSUE WITH RADIOACTIVE IODINE AS INDICATOR

Animal No.	Treatment	Iodide given	Percentage uptake 100 mg.
1	None	Tracer	20.3%
2			16.3%
3	Propylthiouracil	Tracer	.87%
4			.98%

5 mg. of propylthiouracil suspension were injected intraperitoneally 1 hour prior to iodide administration. The rats were killed 1 hour after radioactive iodine, in tracer quantities, was injected subcutaneously.

in the thyroid iodide. The thyroid glands of animals continuously fed propylthiouracil in this experiment averaged 3.1 times the size of the normals or those receiving an injection of propylthiouracil 1 hour prior to the experiment.

Table 3 shows the effect of a single injection of radioactive iodine on the total uptake of radioactive iodine in thyroids of normal animals

TABLE 4. WHITE RATS WEIGHING 38 TO 50 GRAMS ALL GIVEN 0.1% PROPYLTHIOURACIL IN THE DIET FOR 15 DAYS PRIOR TO EXPERIMENT

Animal No.	Additional treatment	Mg. iodine/100 gm. chemical determination	Mg. iodine/100 gm radioactive determination
1	None	3.44	
2			
3		3.32	
4			
		Average 3.38	
5	10 mg. KSCN 1 hr. before killing	0.86	
6			
7		0.87	
8			
		Average 0.86	
9	76 μg. I^- ½ hr. before killing	19.2	17.3
10		21.0	25.9
11		21.8	27.1
		Average 20.7	23.4
12	76 μg. I^- 1½ hr. before killing	21.4	27.2
13		21.6	26.3
14		21.8	30.1
		Average 21.6	27.9
15	76 μg. I^- 1½ hr. +10 mg. KSCN 1 hr. before killing	4.74	1.04
16		0	0.65
17		1.72	1.03
		Average 2.15	0.91
18	76 μg. I^- 1½ hr. +10 mg. KSCN 15 min. before killing	0	1.12
19		1.82	0.83
20		0	0.31
		Average 0.61	0.75
21	76 μg. I^- 1½ hr. +5 mg. KSCN 1 hr. before killing	3.00	0.16
22		0.87	1.55
23		3.64	1.76
		Average 2.50	1.16
24	76 μg. I^- 1½ hr. +1 mg. KSCN 1 hr. before killing	1.31	1.91
25		3.29	0.27
		Average 2.30	1.09

and in thyroids of animals treated one hour previously by the intraperitoneal injection of 5 mg. of propylthiouracil. In the normal animals the radioactive iodine uptake represents iodide accumulated plus iodine in the process of transformation into hormone. The uptake of iodine in the animals receiving 5 mg. of propylthiouracil one hour

previous to the injection of the radioactive iodine was only 5% that of the first group. The difference represents iodine in the process of incorporation into protein. In both groups the iodide concentrating mechanism is unimpaired.

The effect of thiocyanate on the iodide concentrating mechanism: The thyroid glands of rats depleted of iodine under the influence of

TABLE 5. RATS AVERAGING 75 GRAMS ALL GIVEN 0.1% PROPYLTHIOURACIL IN THE DIET FOR 23 DAYS PRIOR TO EXPERIMENT

Animal No.	Additional treatment	Mg. iodine/100 gm. chemical determination	Mg. iodine/100 gm radioactive determination
1	None	3.19	
2		1.32	
3		2.49	
		Average 2.53	
4	1 mg. KSCN 15 min. be-	0	
5	fore killing	0	
6		0	
		Average 0	
7	100 μg. KI 30 min. before	13.3	12.8
8	killing	19.8	16.4
9		22.6	18.9
		Average 18.6	16.0
10	100 μg. KI 45 min. before	22.2	19.3
11	killing	26.8	24.9
12		31.6	29.0
		Average 26.9	24.4
13	100 μg. KI 45 min. +1 mg.	3.28	3.16
14	KSCN 15 min. before kill-	4.78	3.77
15	ing	2.97	3.60
		Average 3.68	3.51
16	100 μg. KI 45 min. +0.3	13.7	10.7
17	mg. KSCN 15 min. before	7.3	6.3
18	killing	8.5	7.1
		Average 9.8	8.0
19	100 μg. KI 45 min. +0.1	9.1	9.2
20	mg. KSCN 15 min. before	23.7	21.2
21	killing	(lost)	7.6
		Average 16.4	12.7
22	100 μg. KI 45 min. +0.03	15.3	12.6
23	mg. KSCN 15 min. before	13.0	13.9
24	killing	9.7	8.2
		Average 12.7	11.6

propylthiouracil have a strong affinity for iodine, which has been shown to behave as iodide. In studying the effect of thiocyanate, rats were prepared with propylthiouracil and 100 micrograms of potassium iodide were injected subcutaneously. Thirty minutes were allowed to elapse, and then varying doses of potassium thiocyanate were injected intraperitoneally and the animals killed 15 minutes to 1 hour afterwards. It was observed that potassium thiocyanate under such

conditions caused a prompt discharge of the recently concentrated iodide. Data on such experiments are presented in tables 4 and 5, graphically shown in figures 3 and 4. It is noteworthy that a dose of potassium thiocyanate as small as 30 micrograms causes a significant lowering of the thyroid iodine concentration after a dose as large as 100 micrograms of potassium iodide and that this has occurred 15

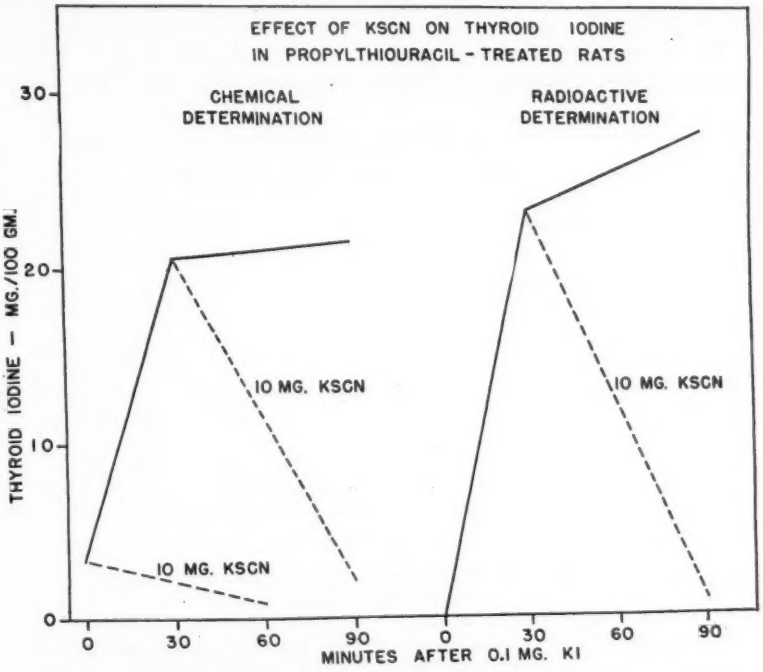

FIG. 3 shows the rapid uptake of iodide by thyroid of rats treated chronically with propylthiouracil. The effect of 10 mg. of potassium thiocyanate on the thyroid iodide concentration is shown.

minutes after the injection of potassium thiocyanate. Since it has been shown that the thyroid maintains an iodide gradient between thyroid cells and serum it is apparent that thiocyanate inhibits this function whether given before or after iodide is administered. In addition it was found possible to discharge labelled radioactive iodine accumulated by the thyroids of normal animals injected with 5 mg. of propylthiouracil 1 hour before radioactive iodine was given.

Further it may be noted that despite prolonged propylthiouracil treatment a concentration of iodine in the range of 5 mg. per hundred grams of thyroid tissue is found. That this is not protein-bound iodine but rather iodide ion was shown previously using the silver-silver iodide electrode. The prompt lowering, practically to zero, which

occurs when a single injection of potassium thiocyanate is given confirms this observation. When potassium thiocyanate is injected into normal rats no detectable effect on the thyroid iodine content is noted; in such thyroid glands the iodine is largely protein-bound.

Potassium bromide has also been tested for ability to discharge iodide under similar conditions. Bromide in amounts over 500 times that of previously injected iodide caused no significant lowering of the iodide level.

To determine the duration of the effect of thiocyanate, rats in groups of three which had been depleted of iodine as described above

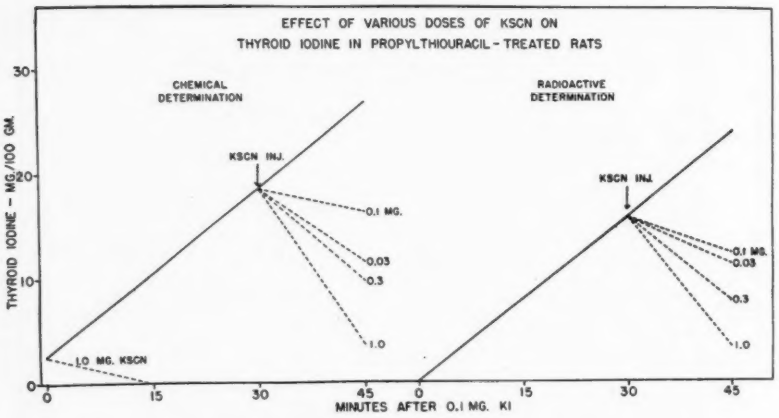

FIG. 4 shows the effect of increasing doses of thiocyanate in causing the discharge of iodide.

TABLE 6. DURATION OF ACTION OF 2 MG. KSCN ON INHIBITION OF IODIDE CONCENTRATION BY THE THYROID

	Mg. iodine/100 gm. thyroid		Mg. iodine/100 gm. thyroid
Group 1. KI	29.5	Group 5. KSCN	3.43
	26.0	KI 2 hrs. later	4.25
	33.3	Average	3.84
Average	29.6		
Group 2. KSCN	0.58	Group 6. KSCN	4.63
	6.48	KI 3 hrs. later	4.85
	0		9.55
Average	2.32	Average	6.34
Group 2. KSCN	3.75	Group 7. KSCN	6.25
KI	1.72	KI 4 hrs. later	11.3
	3.57		3.33
Average	3.01	Average	6.98
Group 4. KSCN	2.86	Group 8. KSCN	14.0
KI 1 hr. later	4.35	KI 5 hrs. later	13.6
	3.98		8.4
Average	3.73	Average	12.0

were all injected with 2 mg. of potassium thiocyanate intraperitoneally except controls which received 100 μg. of potassium iodide only. At hourly intervals from zero to five hours the thiocyanate injected animals received 100 μg. of potassium iodide subcutaneously. All animals were killed 15 minutes after receiving the iodide injection. The results show that in the dosage used the effect of thiocyanate persists for 3 to 4 hours and at 5 hours the effect is definitely less marked. Results are shown in table 6 and figure 5.

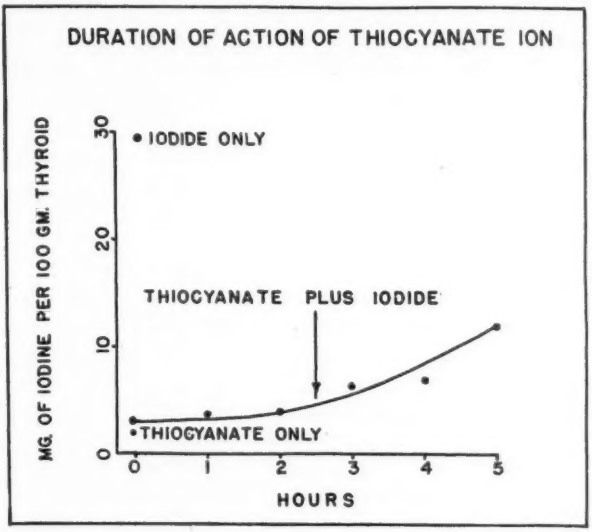

FIG. 5 shows the duration of action of 2 mg. of potassium thiocyanate in inhibiting iodide uptake. 100 micrograms of potassium iodide were given from 0 to 5 hours after thiocyanate was administered.

In view of the marked structural difference between the thiourea type of antithyroid compound and the aminobenzene type rats were fed 0.1% propylthiouracil and 2% sulfaguanidine and the ability to concentrate injected iodide after depletion of hormone was tested. It is clear that the thyroid of the sulfaguanidine treated animal is able to concentrate iodide as does the thyroid of the animal treated with thiouracil type compounds (table 7). This confirms the results of *in vitro* iodide accumulation studies with similar compounds reported by Schachner, Franklin and Chaikoff (1944).

DISCUSSION

The data presented in this paper appear to establish several points. Firstly, under the conditions of the experiment, *iodide* has been demonstrated in large quantities in thyroid tissue. Secondly, the relation of thyroid iodide to serum iodide has been studied, and a

gradient for iodide between thyroid and serum has been demonstrated. Over a wide range of serum iodide concentrations the gradient has been found constant, so that the thyroid iodide concentration is a direct function of the level of serum iodide. In anatomically normal thyroids this iodide concentrating mechanism is able to maintain a twenty-five fold increase of thyroid iodide over serum iodide. A striking result of pretreatment with propylthiouracil is a

TABLE 7. EFFECT OF 0.1% PROPYLTHIOURACIL AND 2% SULFAGUANIDINE ON THYROID WEIGHT AND IODINE CONTENT AFTER POTASSIUM IODIDE INJECTION

	Animal No.	Treatment	Thyroid weight/100 grams of rat	Mg. iodine/100 gm. thyroid tissue
Control	1	None	10.8	69.1
	2	None	10.0	
			Av. 10.4	
Control	3	200 μg. KI 1 hr.	13.8	83.7
	4	before killing	14.7	
			Av. 14.2	
0.1% propylthiouracil 18 days	5		52.4	2.41
	6		52.1	
	7		40.0	2.61
	8		69.5	
			Av. 53.7	
				Av. 2.51
0.1% propylthiouracil 18 days	9	200 μg. KI 1 hr.	53.4	35.1
	10	before killing	57.9	
	11		57.8	28.2
	12		64.1	
			Av. 58.3	Av. 31.6
2% sulfaguanidine 18 days	13		35.1	1.37
	14		48.1	
	15		30.5	4.33
	16		28.9	
			Av. 37.4	
				Av. 2.85
2% sulfaguanidine 18 days	17	200 μg. KI 1 hr.	36.5	25.2
	18	before killing	34.7	
	19		57.3	22.4
	20		60.0	
			Av. 47.1	Av. 23.8

marked increase in the capacity of the thyroid for concentrating iodide such that the ratio of thyroid iodide to serum iodide rises to the value of 250 to 1. Thirdly, this iodide concentrating mechanism appears to maintain a dynamic equilibrium between thyroid and serum, and the preferential concentration of iodide in the thyroid is inhibited by the administration of thiocyanate. Thus thiocyanate is able to cause the discharge of iodide from the thyroid gland as well as to prevent its uptake.

The demonstration that the iodine taken up under the conditions

of these experiments is ultrafiltrable and that it behaves as iodide when studied potentiometrically using the silver-silver iodide electrode provides the most direct evidence thus far presented that iodide itself exists in the thyroid gland.

Although it had long been held that the ability of the thyroid to concentrate iodine depended entirely on its capacity for making hormone, Astwood (1944-45) showed that the processes could be divorced. In these experiments this has been done both with anatomically normal and with hyperplastic thyroid glands by means of the acute and of the chronic administration of propylthiouracil, respectively. Using tracer technique a gradient for thyroid and serum iodide has been found which is constant over a wide serum level of iodide.

In these experiments we have distinguished carefully between iodide uptake and subsequent steps in hormone formation, the first of which involves the binding of iodine to protein. The importance of this can be seen from the data in table 3. Here it is noted that in anatomically normal thyroids acutely inhibited by propylthiouracil, the total uptake of iodine is only 5% that of normal glands not under the influence of propylthiouracil. Thus the ability of the gland to incorporate iodine into an organic form allowed 20 times as much iodine to be taken up. Nonetheless the acutely inhibited glands were able to maintain a high ratio between thyroid iodide and serum iodide (table 2). Thus, whereas the injection of propylthiouracil caused no inhibition of the iodide concentrating mechanism, the failure of such glands to make hormone could readily lead to the completely erroneous conclusions that the effect of thiouracil on iodine metabolism was to inhibit the uptake of iodide instead of the formation of hormone.

The observation that one of the effects of hyperplasia of the thyroid is to increase the thyroid-serum iodide gradient tenfold when considered in the light of the threefold weight increase which occurred with the administration of propylthiouracil indicates an overall thirtyfold increase in the capacity of the entire thyroid gland for taking up iodide. This increased capacity for iodide uptake which occurs with the hyperplasia following the administration of propylthiouracil might occur in other conditions leading to hyperplasia and may be regarded as a compensatory mechanism allowing a more efficient utilization by the thyroid of the amount of iodide available to it. Thus, for example, relative deficiency of iodide occurring both with increased demand for thyroid hormone and with low dietary iodine intake may lead to hyperplasia and thus to a greater efficiency of the iodide concentrating mechanism.

The exact mode of action of thiocyanate remains obscure although it seems clear that thiocyanate prevents the thyroid from maintaining a gradient between thyroid and serum for iodide. It will be important to learn whether or not thiocyanate is concentrated by the thyroid.

In many regards, thiocyanate is known to behave as a halide and its effect may depend on this property. However, it is to be noted that bromide in very large doses had no such effect.

SUMMARY

The observation is confirmed that thyroid depleted of hormonal iodine under the influence of propylthiouracil is still capable of concentrating large quantities of iodine when potassium iodide is injected. This iodine is held in the reduced form as iodide under these conditions. It is entirely ultrafiltrable and all has been shown to be iodide when titrated potentiometrically using a silver-silver iodide electrode. A gradient exists between serum and thyroid iodide concentration. This gradient is decreased only when the serum iodide level is relatively high.

The thyroid serum-iodide gradient increases in the range of ten fold with thyroid hyperplasia, induced by propylthiouracil. The thyroid may hypertrophy to three or more times normal size concomitantly with hyperplasia. This results in a total ability of the stimulated thyroid to accumulate an amount of iodide thirty or more times greater than the amount concentrated by a normal gland in equilibrium with a similar serum iodide concentration.

Thiocyanate inhibits preferential concentration of *iodide* by thyroid tissue. It causes discharge of iodide from the thyroid as well as preventing its uptake. Thiocyanate in the dosage tested still has a partial inhibitory effect on thyroid iodide concentration five hours after injection.

Bromide ion in doses over 500 times that of injected iodide will not cause significant iodide displacement from thyroid tissue.

Sulfaguanidine does not prevent iodide concentration by the thyroid.

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CYTOCHEMICAL EVIDENCE FOR THE CESSATION OF HORMONE PRODUCTION IN THE ZONA GLOMERULOSA OF THE RAT'S ADRENAL CORTEX AFTER PROLONGED TREATMENT WITH DESOXYCORTICOSTERONE ACETATE[1]

ROY O. GREEP AND HELEN WENDLER DEANE

From the Harvard School of Dental Medicine and Department of Anatomy, Harvard Medical School

BOSTON, MASSACHUSETTS

COMBINED morphological and cytochemical studies have revealed that the zona fasciculata of the adrenal cortex may atrophy, become hyperactive, or become exhausted without any conspicuous alteration in the zona glomerulosa (Deane and Greep, 1946; Deane and McKibbin, 1946; Deane and Shaw, 1947). The interpretation placed on these findings is that the adrenal cortex is composed of two separate secreting zones which produce different types of cortical hormones.

Evidence obtained from physiological, chemical, and morphological studies of the adrenal cortex has suggested the dual nature of this gland. Physiologically, the symptoms arising from total loss of cortical tissue arise from two separate disturbances: 1) an inability to regulate sugar and protein metabolism, and 2) a derangement of fluid and electrolyte balance. Chemically, the hormones which have been extracted from the cortex comprise two groups of steroids, distinguishable by the presence or absence of an oxygen or hydroxyl radical on the eleventh carbon of the phenanthrene ring and expressing themselves physiologically in the two separate ways mentioned above. Thus the C_{11}-oxygenated corticosteroids stimulate gluconeogenesis, and the 11-desoxy compounds control sodium distribution (Swingle and Remington, 1944). Morphologically, the adrenal cortex can be differentiated into two distinctive layers on the basis of cell arrangement: the glomerular, or outer zone, and the fasciculate, or columnar, which comprises the remainder of the cortex. These two zones both contain lipid droplets and are separated by a fat-free transitional region.

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[1] This work was done in part under a grant from the American Cancer Society to the Department of Anatomy on the recommendation of the Committee on Growth of the National Research Council.

In the last few years several workers have discussed the possibility that the zona glomerulosa may secrete the 11-desoxy type of corticosteroids and the zona fasciculata the 11-oxy type of cortical hormones. Following hypophysectomy, the rat shows a selective atrophy of the fasciculata and suffers principally from a loss of the cortical sugar factor, while the glomerulosa shows little alteration and the fluid and electrolyte metabolism is less affected. Principally on the basis of these sequelae of hypophysectomy, Swann (1940) proposed that the cortical factor responsible for gluconeogenesis might arise in the "internal layers" through pituitary stimulation, whereas the "portions of the cortex only slightly affected by hypophysectomy, i.e., primarily the glomerular layer, secrete the 'salt and water' hormone" (page 511). Our own analysis of the cytochemical changes in the adrenal cortex following hypophysectomy have supported and elaborated this thesis (Deane and Greep, 1946; Greep, 1946). The observations that adrenal extract and 11-oxygenated corticosterones as well bring about what appears to be a disuse atrophy of the fasciculata in intact rats is consistent with this general concept (Ingle, Higgins and Kendall, 1938), as is also the fact that selective stimulation followed by exhaustion of the fasciculata occurs when animals are subjected to various stresses (Dalton et al., 1944; Popják, 1944; Deane and Shaw, 1947).

The converse experiment to that of Ingle, Higgins and Kendall was carried out by Carnes et al. (1941), Sarason (1943) and del Castillo and Rapela (1945), who injected desoxycorticosterone, an active "salt and water" hormone, to determine what changes would occur in the adrenal cortex. Two mg. or more of the steroid were injected daily into rats for at least a month. There was recorded an atrophy of the cortex, with a complete disappearance of osmiophilic, sudanophilic, and birefringent lipids from the outer border of the cortex but no marked alteration of the lipid content of the inner zones. Similarly, in injected, hypophysectomized animals Sarason obtained a depletion of lipid from the glomerulosa.

Because of the importance of these observations, we have reinvestigated the effect of administration of desoxycorticosterone acetate, using additional cytochemical criteria for determining the ketosteroid content of the two major zones of the adrenal cortex.

MATERIALS AND METHODS

Adult male rats of the Long-Evans strain were used for the experiments. They had been raised in our laboratory and were maintained after weaning on a diet of Purina fox chow.

Ten rats were injected subcutaneously each day with 2 mg. desoxycorticosterone acetate (Percorten, Ciba[2]) in oil as follows: 3 intact animals were

[2] The Percorten was supplied by the Ciba Pharmaceutical Products, Inc., Summit, N. J., through the generosity of Dr. Robert Oppenheimer.

treated for 28 days; 2 rats, hypophysectomized 35 days, for the last 7 days; 2 rats, hypophysectomized 40 days, for the last 12 days; and 3 rats, hypophysectomized 56 days, for the last 28 days. All necropsies were made on the day following the last injection.

Control adrenal glands were obtained from 5 unoperated animals, 5 that had been hypophysectomized for 28 days, and 5 that had been hypophysectomized for 56 days.

In addition, water-soluble desoxycorticosterone-glycoside (Ciba)[3] was studied for short-term effects. Three rats were given injections of a solution containing 5 mg. of the compound (calculated to contain about 2.5 mg. desoxycorticosterone) and 1 rat with 10 mg. The solution was divided into 4 parts and injected at 6 hour intervals. The rats were killed 6 hours after the last injection. Two control rats received at the same time injections of an equal quantity of Ringer's fluid.

At autopsy the paired adrenal glands and the thymus were weighed. The adrenals were fixed in 10% neutralized formalin for at least 48 hours, then washed in running water for an hour and sectioned on the freezing microtome at 15 μ. One of the sections was stained with sudan IV, another with sudan black B, and a third was treated with the Schiff reagent. An unstained section, as well as a control section previously extracted for $\frac{1}{2}$ hour in cold acetone, was examined with the polarizing and fluorescence microscopes to determine the quantity and distribution of acetone-soluble birefringent and autofluorescent materials in the cortex. These several reactions characterize the ketosteroids, and any lipid which reacts in a positive manner by all of them probably contains such substances (Dempsey and Wislocki, 1946).

RESULTS

Average figures for the body weights of the various groups of rats and for the weights of the paired adrenals are presented in Table I. A significant decrease in the proportionate size of the adrenals occurs with desoxycorticosterone acetate treatment in both intact and hypophysectomized rats. The thymus weights, on the other hand, are variable but not significantly abnormal. The lack of any significant effect of desoxycorticosterone on thymus size has been noted by others (Wells and Kendall, 1940; Carnes et al., 1941).

Histology and Cytochemistry of the Adrenal Cortices of the Controls

The histology of the normal rat's adrenal cortex and the distribution of ketosteroids therein have been described elsewhere by ourselves and others. Suffice it to say that lipid droplets displaying the various reactions for ketosteroids occur predominantly in the cells of the glomerulosa and the outer fasciculata (Figs. 1, 2, and 3), with a narrow, lipid-free region between.

Following hypophysectomy the zona fasciculata of the cortex atrophies severely and becomes gradually depleted of its lipids (Figs. 7 and 11). This depletion is essentially complete at 56 days. Con-

[3] We are indebted to Dr. George W. Thorn of the Peter Bent Brigham Hospital, Boston, for the supply of water-soluble desoxycorticosterone.

TABLE 1. THE EFFECT OF DAILY INJECTIONS OF 2 MG. DESOXYCORTICOSTERONE ACETATE ON INTACT AND HYPOPHYSECTOMIZED MALE RATS. TREATMENT OF THE LATTER GROUP WAS BEGUN ON THE 28TH DAY AFTER OPERATION

	Controls		Adrenals	
	Number of rats	Av. Wt., g.	Mg.	Mg./100 g.
Intact	5	205	33.4	16.3
Operated 28 d.	5	88	8.3	9.4
Operated 56 d.	5	105	8.0	7.6

	Desoxycorticosterone—injected				
	Number of rats	No. Inj.	Av. Wt., g.	Adrenals	
				Mg.	Mg./100 g.
Intact	3	28	294	31.7	10.8
Operated 35 d.	2	7	115	8.4	7.3
Operated 40 d.	2	12	106	8.7	8.2
Operated 56 d.	3	28	152	7.0	4.6

trariwise, the cells of the glomerulosa retain their full complement of lipid and the reactions for ketosteroids appear, if anything, more intense than normal. Although the individual cells of the glomerulosa diminish somewhat in size, this zone becomes broader, primarily because it maintains its previous volume on the surface of a shrinking sphere, but possibly also because there is some proliferation of the cells (Crooke and Gilmour, 1938; Deane and Greep, 1946).

PLATE 1

Photomicrographs of adrenal cortices of intact adult male rats. Glands fixed in 10% neutralized formalin; frozen sections cut at 15μ. The boundaries of the cortex have been drawn in. All photomicrographs ×90.

FIGS. 1–3. Control rats; Figs. 4–6, rats which had received 2 mg. desoxycorticosterone acetate daily for 28 days. The latter pictures illustrate the slight atrophy of the cortex which occurs with the hormone.

FIG. 1. Normal cortex, stained with sudan IV and hematoxylin; photographed with a blue filter. The sudanophilic lipids occur predominantly in the zona glomerulosa and in the outer part of the zona fasciculata. The lipid-free transitional zone is conspicuous.

FIG. 2. Normal cortex, unstained and photographed on the fluorescence microscope with the aid of a yellow filter. Fluorescent material occurs in all the parenchymal cells of the cortex, and is acetone soluble.

FIG. 3. Normal cortex, unstained and photographed on the polarizing microscope. Mixed coarse and fine birefringent particles occur in the glomerulosa and throughout the fasciculata, with a transitional zone between.

FIG. 4. Cortex of rat receiving hormone, stained with sudan IV and hematoxylin. The glomerulosa is completely devoid of sudanophilic lipid, while the atrophied fasciculata contains more lipid than normal.

FIG. 5. Cortex of rat receiving hormone, unstained and photographed on the fluorescence microscope. The glomerulosa exhibits no autofluorescence.

FIG. 6. Cortex of rat receiving hormone, unstained and photographed on the polarizing microscope. All birefringent material has disappeared from the zone glomerulosa, while that in the fasciculata is somewhat more crowded than in Fig. 3.

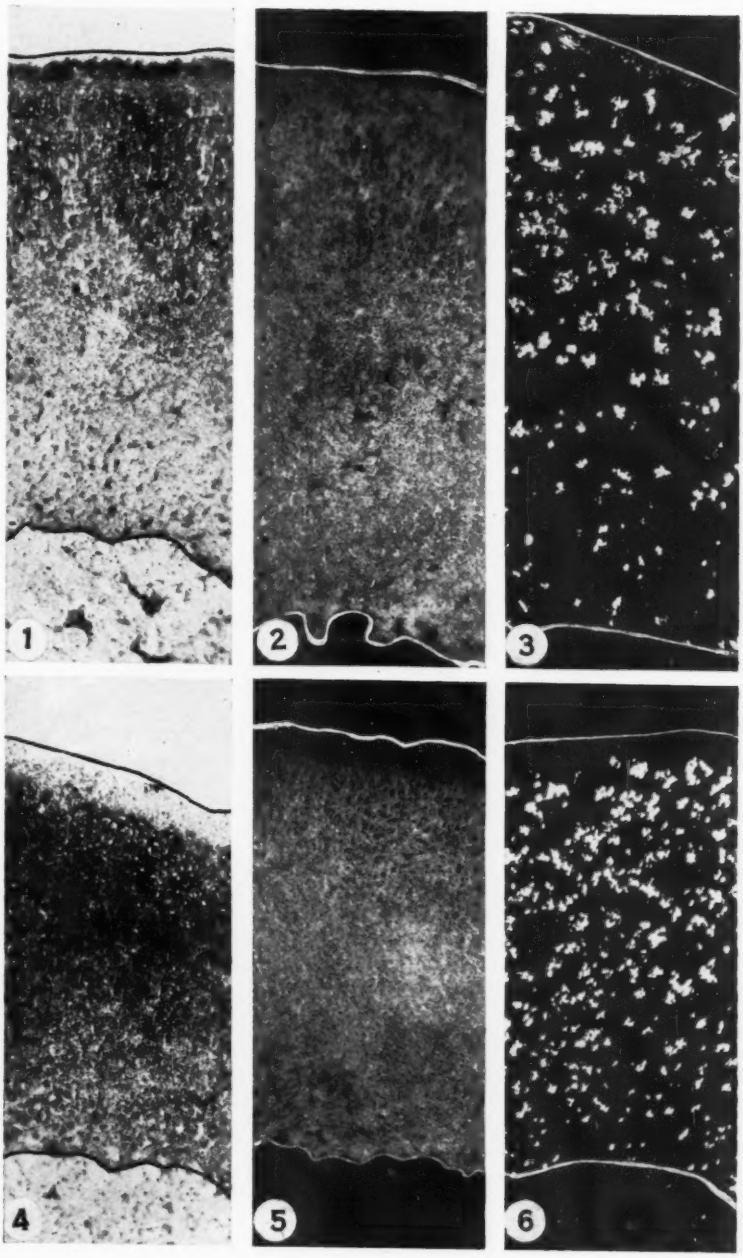

PLATE 1

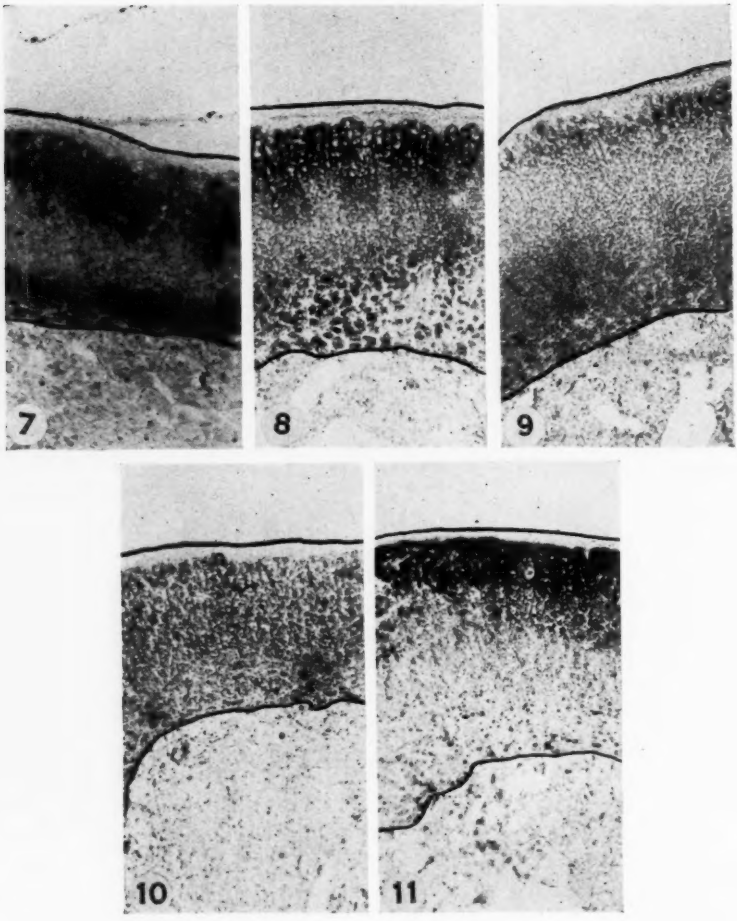

PLATE 2

Photomicrographs of the adrenal cortices of rats which had been hypophysectomized. Glands fixed in 10% neutralized formalin; frozen sections cut at 15μ. All stained by the Schiff method and photographed with combined green and yellow filters. The boundaries of the cortex have been drawn in. ×90.

FIG. 7. Cortex of an untreated rat which had been hypophysectomized for 28 days. There has been a marked atrophy of the inner zones, while the outer zone derived from the glomerulosa is broader than in the intact animal. Schiff-positive lipids occur in the outer and inner regions, with a broad lipid-free zone between.

FIG. 8. Cortex of a rat hypophysectomized 35 days which received 7 injections of desoxycorticosterone acetate, beginning on the 28th day after operation. The normal disappearance of Schiff-positive material from the inner zones has occurred, but in addition there has been a decline in the intensity of the Schiff reaction in the glomerulosa.

FIG. 9. Cortex of a rat hypophysectomized for 40 days which received 12 injections of desoxycorticosterone acetate, beginning on the 28th day after operation. Schiff-

Adrenal Cortices of Rats Injected with Desoxycorticosterone

In describing the changes occurring in the cortex following long periods of injection of desoxycorticosterone acetate, the intact and hypophysectomized animals will be considered together, since the changes produced are essentially identical.

After 7 days treatment of hypophysectomized rats, the zona glomerulosa appears normal in the sudan and birefringence preparations. There is, however, a detectable decrease in both the Schiff reaction (Fig. 8) and in the autofluorescence. No unusual features are noted in the zona fasciculata. After 12 days treatment of hypophysectomized rats the amount of sudanophilic lipids in the glomerulosa is distinctly diminished, the birefringent particles of the zone are sparser and also coarser than before, the autofluorescence is further reduced, and the Schiff reaction is almost completely negative (Fig. 9).

When the injections were continued for 28 days in both intact and hypophysectomized rats, the zona glomerulosa is found to be completely devoid of lipids. Furthermore, a distinct atrophy of the cortex is apparent. While this is difficult to localize in the hypophysectomized animals because of the complete absence of lipid (Fig. 10), in the unoperated animals the atrophy appears to involve principally the zona fasciculata, (Figs. 4, 5 and 6) and perhaps because of shrinkage, the lipid droplets in this zone are more densely crowded than normal (Figs. 4 and 6).

DISCUSSION

The progressive disappearance of ketosteroids from the zona glomerulosa of the adrenal cortex in rats treated with desoxycorticosterone acetate seems most likely to be an expression of a disuse atrophy caused by artificially supplying an abundance of a hormone which operates through the same physiological channels as the natural product of the glomerulosa[4]. Since this response occurs in both intact and hypophysectomized animals; it does not seem probable that it

[4] Despite the fact that progesterone has desoxycorticosterone-like activity (Cantarow and Rakoff, 1940), injections of 2 or 6 mg. of this compound (generously supplied by the Schering Corp.) daily for 30 days did not cause depletion of the glomerulosal lipids in normal or in hypophysectomized rats. It would seem, therefore, that the alterations reported here are remarkably specific for desoxycorticosterone.

positive lipid has disappeared from the inner zones and has virtually disappeared from the outer zone. Moreover, the outer zone containing lipid is narrower than normal.

FIG. 10. Cortex of a rat hypophysectomized for 56 days which received 28 injections of desoxycorticosterone acetate, beginning on the 28th day after operation. Schiff-positive lipid is entirely absent from the cortex.

FIG. 11. Cortex of an untreated rat hypophysectomized for 56 days. By this time the inner zones are normally devoid of Schiff-positive material but the broad outer zone contains a large amount.

involves the pituitary, as appears to be true of corticosterones. This conclusion agrees with earlier suggestions that the glomerulosa is not a conspicuous target organ of the pituitary (Sarason, 1943; Deane and Greep, 1946; Ingle, Li and Evans, 1946).

The nature of the mechanism which regulates the glomerulosa is totally unknown. We only state the problem when we suggest that desoxycorticosterone maintains the normal equilibrium of some substance, possibly sodium, which, if at a subnormal level, stimulates glomerulosal secretion. This possibility is strongly supported by the recent study of Knowlton et al. (1947). These investigators found that whereas glomerulosal depletion and atrophy occurred in rats on a normal diet when receiving 2.5 mg. desoxycorticosterone acetate daily, such changes did not occur in similarly injected rats which were fed a diet low in sodium. Apparently until the concentration of sodium in the blood reaches a certain level, secretion by the glomerulosa can not be cut off.

We wish to emphasize that the gland can be depleted of its secretory product and the precursors thereof by two processes which represent diametrical extremes. In atrophy accompanying the injections of desoxycorticosterone, as observed here, or the injections of 11-oxy-corticosteroids, as well as after hypophysectomy, the glandular tissue shrinks and there is a slow leaching out of the demonstrable lipids and steroids. This type of exhaustion takes several weeks. On the other hand, in acute hyperfunction there is an hypertrophy of the glandular parenchyma accompanied by a rapid disappearance of the products of secretion. In this type of depletion all ketosteroid reactions may disappear from the fasciculata in less than a day (Deane and Bergner, 1947). Failure to consider these two types of depletion continues to lead some workers to misinterpret the intensity of the secretory stimulus that is acting on a given gland.

On the basis of our hypothesis of the duality of secretory zones in the cortex, however, it is difficult to explain the atrophy which occurs in the zona fasciculata. Since this atrophy is accompanied by a slight concentration of lipid droplets, there appears to be some suppression of the secretion of the adrenocorticotropic hormone when a high level of desoxycorticosterone is artificially supplied. The observations of Sayers and Sayers (1947) support this suggestion, since they found that desoxycorticosterone was one-eighth as active as some 11-oxy-corticosteroids in preventing the release of adrenotropin. However, the reason for the accentuated atrophy of the cortex in hypophysectomized rats receiving desoxycorticosterone is not apparent.

SUMMARY AND CONCLUSIONS

Injections of 2 mg. desoxycorticosterone acetate daily for 28 days completely inhibit the production of ketosteroids in the cells of the zona glomerulosa of the rat's adrenal cortex. This suppression occurs

both in intact and in hypophysectomized rats. A reduction in the autofluorescence and in the Schiff reaction of the glomerulosal lipid is detectable after 7 days of treatment; by 12 days there is a further loss of both autofluorescence and the Schiff reaction, a reduction in the amount of birefringence and a narrowing of the lipid band. This depletion of lipid from the glomerulosa is interpreted as a disuse atrophy produced by the exogenous supply of a hormone which can substitute for the normal products of this zone.

A slight atrophy occurs also in the zona fasciculata following desoxycorticosterone injection. This should probably be attributed to the suppression of secretion of the adrenocorticotropic hormone.

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THE EFFECT OF PHENOBARBITAL ON THE ACTION OF INSULIN

J. M. JOHLIN

From the Department of Biochemistry, Vanderbilt University, School of Medicine

NASHVILLE

THE OBJECTS of the present experiments were (1) to determine the effect of phenobarbital on the blood sugar level of insulinized rabbits, (2) to observe the tendency of such animals to go into convulsions, and (3) to note if the administration of insulin to rabbits altered their response to electrical stimulation.

No previous observations on the first and second of these objectives appear to have been reported. Waltner, (1925) found that an increased response in irritability to the stimulating influence of the cathode closing current runs parallel to the decreased level of blood sugar produced by insulin in infants and children. Mann, (1934) found that, while insulin increased the tendency to tetany of a lead damaged individual, neither insulin nor the administration of excessive amounts of glucose affects the muscular rheobase or chronaxie in normal individuals nor the tendency to tetany in postoperative cases, and that the latent tendency to tetany in parathyroidectomized dogs is independent of the level of blood sugar produced by insulin.

Each animal which was used in the present experiments had been carefully standardized to determine the number of units of insulin per Kg. of body weight which would consistently produce convulsions when injected subcutaneously into the fasting animal. The amount which consistently just failed to produce convulsions was chosen as the subconvulsive dose. Food was witheld from experimental animals for 18 hours prior to an experiment. The insulin used was a commercial grade of plain insulin (Squibb) and was administered subcutaneously. Solutions of sodium phenobarbital (Na P B) were also injected subcutaneously and were freshly prepared a few minutes earlier. When both insulin and phenobarbital were used in the same experiment, the injection of insulin was given an hour after that of the barbital. Blood sugar determinations were made according to the method of Van Slyke and Miller, (1936). Representative data are given in Table I.

The data of group A are in accord with the known fact that barbiturates, when injected into the normal animal, do not change the level of the blood sugar.

The data of group B were obtained with a second group of animals

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which had been injected with a subconvulsive dose of insulin only. The data of group C were obtained with the same animals after they had been injected with 60 mg. of Na P B per Kg. of body weight, followed by a subconvulsive dose of insulin.

The data of groups D and E were obtained with a third group of animals under similar conditions as those of groups B and C and confirm the nature of the effects indicated there. The data show that Na P B, when used in conjunction with insulin, increases the effect which insulin has in lowering the level of the blood sugar and prolongs

TABLE I. ILLUSTRATING THE EFFECT OF THE INJECTION OF SODIUM PHENOBARBITAL AND INSULIN UPON THE LEVEL OF THE BLOOD SUGAR OF RABBITS

Experimental Group	Number of Animals	Dose of NaPB per Kg. of body weight	Dose of Insulin	Intervals at which level of blood sugar was determined (hours)						
				0	1	2	3	4	5	6
A	9 (Group I)	60 mg.	none	100	99	101	97	97	98	99
B	8 (Group II)	none	S.C.D.*	105	50	41	51	65	88	98
C	8 (Group II)	60 mg.	S.C.D.	103	48	37	35	50	71	74
D	9 (Group III)	none	S.C.D.	97	50	41	46	59	76	86
E	9 (Group III)	60 mg.	S.C.D.	95	50	35	36	38	51	72
F	9 (Group III)	10 mg.	S.C.D.	92	43	32	35	44	55	70
G	9 (Group III)	10 mg.	S.C.D.	98	46	36**	51	55	60	62

* S.C.D.—Subconvulsive dose.
** Injection of adrenalin. Level of blood sugar was found unchanged 15 minutes later.

it. The effect of Na P B in thus lowering the blood sugar was not as pronounced in some animals as it was in others where the level reached was considerably below that of the convulsive level produced by insulin alone. The average convulsive level found in the process of standardization of these animals was 31 mg. per cent, varying among individuals by ± 3 mg. per cent. After a subconvulsive dose of insulin subsequent to the administration of Na P B, blood sugar levels as low as 17 and 18 mg. per cent were to be found. The time during which individual animals reached the lowest level of blood sugar varied from 2 to 5 hours after the injection of insulin.

The animals used in group E went into convulsions of the clonic type, a condition which was in some cases accompanied by occasional squealing. Of two animals, not included in this group, subjected to similar treatment, one died during the experiment and the other about 24 hours later. Neither had been given injections of glucose during the experiment. The blood sugar level of the former had decreased to 18 mg. per cent and the lowest level attained by the latter, 5 hours after the injection of insulin, had been 23 mg. per cent. The

blood sugar of the latter had returned to a normal state on the following day but the animal had continued to remain in a highly nervous state.

Four of the animals used in group E were injected with 20 mg. of Na P B per Kg. of body weight, followed by a subconvulsive dose of insulin. All went into clonic convulsions, reaching blood sugar levels as low or lower than those attained at the convulsive level during the process of standardization. A fifth animal, which was similarly treated but is not included among those used in group E, remained in convulsions for seven hours and appeared to recover to a normal state after being given injections of glucose at that time but collapsed rather suddenly 15 hours later and died.

The data of group F were obtained with the 9 animals used in group E after they had been injected with 10 mg. Na P B per Kg. of body weight, followed by a subconvulsive dose of insulin. Four of these animals went into mild convulsions, which more nearly resembled those of the tetanic type, and recovered without receiving glucose injections. A fifth animal whose insulin tolerance was unusually high, its normal subconvulsive dose being 3.8 units per Kg. of body weight, remained in a convulsive state for 6 hours and acquired a blood sugar level below that of the normal convulsive state, but recovered immediately when given injections of glucose to bring it out of its state of collapse.

These 9 animals were then re-tested with subconvulsive doses of insulin to note any possible change in their insulin tolerance. None of them gave evidence of a decreased tolerance.

The data of group G were obtained after the 9 re-tested animals were again injected with 10 mg. of Na P B per Kg. of body weight, followed by the injection of a subconvulsive dose of insulin. Two hours after the injection of the insulin, each animal also received a subcutaneous injection of 0.01 cc. of 1/1000 adrenalin per Kg. of body weight. An additional sample of blood was taken 15 minutes after the injection of the adrenalin for the determination of its level of glucose. The adrenalin had the immediate effect of bringing the animal out of its somewhat droopy state to a normal posture but did not during that 15 minute interval increase the level of its blood sugar above that found just previous to the injection of the adrenalin, nor did it in all instances protect the animal from subsequent convulsions. One animal went into mild convulsions followed by a state of prostration from which it recovered immediately to a normal state following the injection of glucose.

Three of these animals were later injected with amounts of insulin which exceeded the convulsive dose by 20 per cent. Adrenalin administered as above when the convulsive state was reached had little apparent effect in preventing repeated convulsions or in raising the level of blood sugar. One of these animals, which had an abnormally high insulin tolerance, failed to return to its usual normal state after

the injection of glucose. For several succeeding days it appeared to be in a demented state, leaping about and dashing into the sides of its cage when approached, and struggling and squealing when handled. It gradually returned to its normal docile state in about 6 weeks.

Previous to beginning the above experiments, another group of 13 animals was similarly standardized, to determine the amount of insulin which would just fail to produce convulsions, in order to study the effect which a reduced level of blood sugar, approaching that of the convulsive state produced by insulin, might have upon the threshold of response to stimulation by a galvanic current applied to a suitable motor point on the skin. At no stage of an altered level of blood sugar of any animal was the threshold of either the cathodal or anodal closing contraction found to differ from that found in the case of the normal animal when stimulated by a galvanic current drawn from a 45 volt battery for a period of 10 seconds.

Six of the surviving animals which had been used in obtaining the data of group G were subsequently used to determine the effect, if any, which a lowered level of blood sugar, approaching that of the convulsive state produced by insulin, might have upon the convulsive threshold to stimulation by a faradic current through the brain of the animal (from the occiput to the mouth) for a period of 10 seconds. The current from a 45 volt battery was interrupted 60 times a second so as to produce a rectangular current of equal periods of flow and interruption. The number of milliamps required to produce convulsions in the normal animal was in each case approximately the same as that when the animal had been brought near the convulsive state through insulin.

SUMMARY

The administration of both sodium phenobarbital and subconvulsive doses of insulin to rabbits causes the animals to convulse, lowers the level of blood sugar below that produced by subconvulsive doses of insulin alone, and prolongs the return of the level of blood sugar to a normal state.

The administration of insulin to rabbits in sufficient amounts to bring these animals near the convulsive state does not alter their threshold of response to the stimulating action of a galvanic current applied to a suitable motor point on the skin, or that to the stimulating action of a faradic rectangular current flowing from the occiput to the mouth.

ACKNOWLEDGMENT

The author is greatly indebted to the donors of the Walter C. Hadley Fund for the aid given these experiments

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NOTES AND COMMENTS

SOME OBSERVATIONS ON PROGESTERONE ANESTHESIA IN THE RAT[1]

SELYE (1, 2) has demonstrated (a) the anesthetic effects of physiologically active steroid compounds; (b) the increasing tolerance to the anesthetic effects of steroid hormones which rats develop upon repeated dosage; and (c) the additive anesthetic effets of combinations of steroids.

That the Selye phenomenon constituted real anesthesia was taken for granted, for it is possible to perform an abdominal operation on an animal thus treated exactly as if the animal had been treated with nembutal or ether. However, Farson, Carr and Krantz (3) criticize two of Selye's conclusions, namely: (1) that the effect of large doses of steroids is one of "depression" and does not constitute true "anesthesia," since afferent pathways are not completely blocked (in animals that survive); and (2) that cholesterol, which is not a hormone, also brings about profound depression (when injected intravenously dissolved in polyethylene glycol!). The latter criticism carries its own answer, especially since the intravenous technique differs too profoundly from Selye's method of intraperitoneal administration.

The first criticism of the Maryland workers would seem to be met by the following observations on the abolition of brain potentials of rats under progesterone anesthesia in comparison with rats under the commonly employed anesthetizing drugs. The method developed by one of us (W. E. B., 4–7) was employed. The test is carried out as follows: As soon as the animal is limp from the injection, the brain and the gastrocnemius muscle are exposed. One non-polarizable electrode is applied in the region of the motor area of the brain, the other to the muscle, a galvanometer being interposed to measure the amperage of the difference in potential. In a rat just coming out from under an ether anesthesia the potential is of the magnitude of about 0.125 microamperes, the brain being negative with reference to the muscle. With resumption of anesthesia the potential approaches zero or (especially with CO_2 or chloroform) even reverses and the brain becomes actually positive.

The 6 rats tested in this way while under the anesthetic action of progesterone behaved in the same way except that in none of them did complete reversal occur. This happens sometimes when ethyl alcohol is given. We do have human testimony that the unconscious stage is actually reached with this drug and that painless operations are possible on persons anesthetized by alcohol; hence, there is no reason to presume that a rat in progesterone anesthesia is conscious, albeit unable to respond to pain stimuli.

It is, of course, true that non-specific factors, such as extreme physical

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[1] The generous contribution by Dr. E. Oppenheimer, Ciba Pharmaceutical Products, Inc., of Lutocylin Ciba (progesterone) and Ovocylin P Ciba (estradiol diproprionate) is gratefully acknowledged.

exhaustion may also reverse the brain potential (6) even before consciousness is lost. Nevertheless, we see no reason for not regarding steroid anesthesia as such and favor retaining the word anesthesia with reference to it.

In another series of experiments, Selye's experiments on the acquisition of tolerance to the steroid were repeated in order to determine whether an estrogen, administered after the acquisition of a tolerance to progesterone,

TABLE 1. EXPERIMENTS ON 13 RATS. DEPTH OF ANESTHESIA INDICATED BY: −, NO EFFECT; +, QUIESCENCE; ++, LOSS OF RIGHTING, BODY FLACCID; +++, DEEP ANESTHESIA, WITH MINIMAL RESPONSE TO PAIN STIMULI

RAT	DAYS	1		2		3		4		5		6		7		8			9			10		
	WT. IN GM.	MG PROGESTERONE	ANESTHESIA	MG PROGESTERONE	ANESTHESIA	MG PROGESTERONE	ANESTHESIA	MG PROGESTERONE	ANESTHESIA	MG PROGESTERONE	ANESTHESIA	MG PROGESTERONE	ANESTHESIA	MG PROGESTERONE	ANESTHESIA	γ ESTRADIOL	MG PROGESTERONE	ANESTHESIA	γ ESTRADIOL	MG PROGESTERONE	ANESTHESIA	γ ESTRADIOL	MG PROGESTERONE	ANESTHESIA
1	120	20	++	22	+++	20	++	20	++	20	+	20	+	20	+	10	20	−		20	−	500	19.5	−
2	112	17	+++	15	+++	15	++	15	++	15	+	15	+	15	+		15	−		15	−	500	14.5	−
3	113	15	−	18	+++	18	+++	18	+	18	+	18	+	18	+	1	18	−		18	−	500	17.5	−
4	110			15	+++	15	++	15	+	15	+	15	+	15	+	1	15	−		15	−	500	14.5	−
5	120			10	+++	10	+	15	++	15	+	15	+	15	+	10	15	−		15	−	500	14.5	−
6	120																		100	15	−			
7	124																		500	18	+++			
8	122																		995	20	+++			
9	194																					500	14.5	+
10	170																					500	9.5	+
11	180																					500	5	−

would reduce the tolerance and render the animal again more or less sensitive to progesterone; in other words, to determine if, under such conditions, estrogen and progesterone were antagonistic or additive.

The following 3 series of experiments were performed (by M. R. D.); the results are summarized in the table. In all cases normal young adult albino rats were used. The first group (Nos. 1–5 of table), having an average weight of 115 g., were subjected to a 7-day pre-treatment period involving a daily average dose of 16.6 mg. of progesterone in peanut oil administered intraperitoneally. At the end of this time, when the animals showed a tolerance toward the anesthetic effect of the progesterone, a variety of dosages of estradiol (maximum about 500 γ) in addition to the standard amount of progesterone were injected. The estradiol was administered twice during 3 days, resting the animals on the 9th day, as shown in table. No increase in sensitivity toward the progesterone was apparent.

In the second series of experiments (Nos. 6–8 of table) 3 non-pre-treated rats with an average weight of 122 g. were given heavy intraperitoneal in-

jections of estradiol followed immediately by a large dose of progesterone. Up to 995 γ of estrogen followed by 20 mg. progesterone was used. The results demonstrate only the usual synergism to be found between 2 steroid hormones.

The third group of non-pre-treated rats (Nos. 9–11 of table) had a higher average weight than did the other groups, namely, 181.3 g. Large amounts of estradiol (500 γ) in oil were injected, followed by minimal doses of progesterone. Little or no anesthetic effect was observed.

It was found that estrogens appear to interact synergistically with progesterone in the production of a steroid hormone anesthesia. This is in accordance with the theories propounded by Selye.

One question, however, remains: would a small daily dose of estrogen *hasten* the recovery of sensitivity to the anesthetic action of progesterone after attainment of complete refractoriness, just as a priming with estrogen is necessary before progesterone is able to "unbolt" the sex behavior of a female rat or guinea pig?

CARL G. HARTMAN, W. E. BURGE, AND MARTIN R. DOCTOR
The University of Illinois
Urbana

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NEW BOOKS

BIOCHEMISTRY OF CANCER. By Jesse P. Greenstein, PhD. Academic Press, 1947.

This timely book deals with the induction of tumors, attempts to influence the induction and growth of tumors, and a description of the chemical properties of tumors.

In Chapter III reference is made to 20-methylcholanthrene as "one of the most active and most widely used compounds in experimental carcinogenesis." The author mentions only its first preparation by degradation of bile acids. As a matter of fact all the experimental work with methylcholanthrene in America has been done with synthetic material made available by the development of an efficient synthesis in the Harvard chemical laboratories. This is an achievement which would justify at least a reference to those who were responsible for it. He later states,

"These newer hydrocarbons, tested by Shear for carcinogenicity in mice, added further information on the relation of chemical structure to carcinogenic activity in this species. This information, however, was limited in interest to the chemical nature of the class of compounds studied. Of greater future usefulness was the knowledge which emerged from the use of only a few of these molecules applied to selected biological problems."

The reviewer was unable to find the more "useful knowledge which emerged" in later sections of the book. The final chapter on the usefulness of any of this knowledge has not been written and the possibility comes to mind that the relation of chemical structure to carcinogenic activity will prove of great value in future developments in chemotherapy.

It is also stated,

"The mechanism of carcinogenesis is yet obscure and, although it had been hoped that a study of chemical structure in relation to carcinogenic activity would be enlightening in this respect, it must be admitted that since both polycyclic hydrocarbons and azo dyes produce lung tumors, and since both azo dyes and carbon tetrachloride produce hepatomas, the sharp difference in potency of isomers of methylcholanthrene and the sharp difference in potency of isomers of aminoazotoluene no longer seems crucial."

The reviewer feels that the physico-chemical differences between such classes of compounds may not be as great as the structural formulas would suggest. In addition it is possible for two quite different classes of drugs to accomplish the same end result by affecting different points in the total mechanism. Some examples of this are: hypertensin and pressor amines in raising blood pressure, curare and prostigmin in producing muscle weakness, and heparin and dicoumarol in decreasing coagulability of blood. The author goes on to state,

"This does not, however, imply that the carcinogenic mechanism is not a relatively specific process. Indeed, no reaction of any kind occurs unless the reactants bear some specific relation to each other. Many workers, impressed and perhaps discouraged by the wide variety of carcinogenic agents, have often fallen back upon such vague terms as 'non-specific irritation,' or 'injury' to describe the mechanism of carcinogenesis.

Yet to describe a reaction as nonspecific is a contradiction in terms and has no scientific meaning. The mere fact that not all chemicals, but only a few, will produce cancer, even though these few may be apparently unrelated, is sufficient to establish the carcinogenic process as being due to relatively unique chemical reactions. The nature of these reations is simply not known at the present time."

The reviewer is in full accord with these statements and considers the point exceptionally well put.

In Chapter VI on endocrinology the discussion of human prostatic carcinoma was particularly interesting. Dr. Greenstein pointed out that,

"Estrogen administration in large amounts results in pituitary inhibition and testicular atrophy and, thus, the use of this kind of hormone at sufficiently high levels is, in effect, a chemical castration. It will be noted that underlying the above assumption is the further assumption that the malignant prostate cells are not fully, if at all, autonomous, e.g. they are still controlled to some measure by the same body mechanisms which control the normal prostate cells. . . . On the other hand, it is also possible that cases of the disease which recur after a period of favorable response, or which are refractory from the very start, may actually be in a largely autonomous state and independent of mechanisms of normal body control. . . . No one has yet succeeded in inducing in laboratory animals the adenocarcinoma of the prostate similar to that observed in man, although a squamous cell carcinoma with skeletal metastases has been induced in rats by implantation of methylcholanthrene. The fact that this induced tumor could be transplanted to, and would grow in, female rats accentuates its difference from the glandular tumor in man which is so responsive to estrogen."

Since metastasis has always been considered strong evidence of autonomy of a tumor, his evidence for considering human prostatic carcinoma only partially autonomous would appear to be a novel and important point of view.

Some of the author's comments on chemotherapy in Chapter VII are provocative. He appears to have an antithesis for an empirical search through the "Chemical Formulary" for agents that might affect tumor growth. To be sure the number of known chemicals has become so vast that any such approach to the cancer problem presents too formidable a task to be undertaken by ordinary university or hospital departments. This approach would admittedly be dull but not necessarily fruitless. It was such an approach that led to the discovery of sulfanilamide and related compounds.

The author points out that the field of chemotherapy of cancer has long been a fertile ground for quackery, and as if fearful of spending too much time with this field he briefly skims through the chapter touching upon a few leads being pursued at the National Cancer Institute.

The reviewer considers a serious omission from this chapter the recent work of Haddow and his collaborators in England on the inhibition of tumor growth by carcinogenic hydrocarbons and structurally related substances.

The greater and more important part of the book deals with the enzymatic properties of tumors, a field in which the author has made many and important contributions. The organization and presentation of this material is very readable and offers an exciting and enjoyable experience for those who are interested in the tumor problem. Although the reviewer found himself in disagreement with some of the points of view expressed in the book, he nevertheless found them all interesting and stimulating. Dr. Greenstein's book is a valuable presentation of data and will prove of inestimable help, particularly to those interested in the experimental aspects of the cancer problem.

ARNOLD M. SELIGMAN, M.D.

RECENT PROGRESS IN HORMONE RESEARCH. Proceedings of the Laurentian hormone conference. Volume I. Edited by Gregory Pincus. $7.50. Pp. 399. Academic Press. Inc., New York.

For several years a Hormone Conference of the AAAS has been held annually, first at Gibson Island in 1943 and subsequently at various sites in the Laurentians. There has been considerable interest in publishing the proceedings of this conference since it has been attended with enthusiasm by the participating investigators. The present volume represents the first published report of the conferences. It forms the opening number of what is hoped to be a continuing series reflecting the current status and foreshadowing the future shape of endocrine research.

This first volume consists of 13 papers divided among four principal subjests. Neurohumoral relationships are treated by David Nachmansohn and Frank A. Beach. The Chemistry and Physiology of Adrenal Hormones is the subject of essays by Edward C. Kendall, T. F. Gallagher, C. N. H. Long and Gregory Pincus. The role of hormones in metabolic processes is considered by Leo T. Samuels, Charles D. Kochackian and W. V. Gardner. Finally, Aspects of Clinical Endocrinology is the subject of discussions by Ira T. Nathanson, Fuller Albright, Nathan B. Talbot and Edna H. Sobel, and Arthur Grollman.

An especially valuable aspect of the book is the inclusion of the discussion, recorded by stenotype, which followed the presentation of each paper. These sections of the book have been extraordinarily well edited so that to the readers who know the speakers well, something of their inflections may be recaptured and yet the statements are nicely rounded and read well. Such editing is no easy task; the stenotype copy of extemporaneous remarks is frequently a sorry object indeed. But well done, as it is here, such a record preserves the sparkle of conversation and extends the usefulness of the small conference.

Recent Progress in Hormone Research is a noteworthy addition to the literature of Endocrinology. It should take its distinguished place among the volumes and periodicals devoted to reviews.

EDWARD W. DEMPSEY

ABSTRACTS OF PAPERS

Presented at the Twenty-Ninth Annual Meeting Atlantic City

1. Studies in Corpus Luteum Function.

J. S. L. Browne, J. S. Henry, and E. H. Venning. From the McGill University Clinic, Royal Victoria Hospital, Montreal, Canada, and the Department of Obstetrics and Gynecology, McGill University.

Sodium pregnanediol glucuronidate was determined by the method of Venning and correlated with endometrial biopsies. In 21 cycles in 20 cases biopsies showed no progestational changes when taken just before the onset of the menstrual period, and the pregnanediol in the same cycle was absent. In 45 cycles in 36 cases the biopsies showed early or late progestational changes and this was well correlated with the time of onset or the presence of pregnanediol. On no occasion was the pregnanediol positive in a cycle in which the biopsy showed no progestational change. There were no cases of adrenal tumours with amenorrhoea in this series; in such cases pregnanediol has been found to be positive with an atrophic endometrium. On 10 cycles in 8 cases the biopsies showed progestational changes but no pregnanediol was present in the same cycle. Some of these discrepancies are explicable on the grounds of very large volumes of urine which prevented the detection of pregnanediol. The effect of chorionic gonadotropin on pregnanediol excretion has been further studied. If chorionic gonadotropin is administered in a dose of 1000 international units daily in the early part of the luteal phase, in some instances a marked increase in the excretion of oestrogen occurs (up to as high as 3800 units in one case). The excretion of pregnanediol is prolonged and endometrial biopsies show histological changes similar to those seen in early pregnancy. In certain cases however, the corpus luteum function cannot be prolonged by the administration of this dose.

2. Progesterone Therapy of Uterine Fibromyomata.

Albert Segaloff, John C. Weed (by invitation), and William Parson. From The Alton Ochsner Medical Foundation, The Ochsner Clinic and the Departments of Medicine and Gynecology, Tulane University of Louisiana School of Medicine, New Orleans, Louisiana.

Patients with uterine fibromyomata have been treated with progesterone.

Only those patients in whom it has been possible to outline the tumor by roentgenography with either intraperitoneal carbon dioxide or the intrauterine instillation of radiopaque oil have been followed.

Three patients were treated for 39 to 61 days with daily intramuscular injections of 20 mg. of progesterone. One patient was given 100 mg. of anhydrohydroxy progesterone by mouth daily for eight weeks.

All four of these patients have come to surgery. There was no decrease in the size of the fibromyomata. Many of the fibromyomata showed microscopic evidence of regression.

Three additional patients have been implanted with 7 or 8 200 mg. pellets of progesterone. They have been followed from one to six months but none have as yet come to surgery.

There has been no significant interference with the menstrual cycles. Urinary gonad stimulating hormone and 17-ketosteroid excretion have also not deviated significantly from the pretreatment values.

3. Distortion of the Spiral Artery in the Ovary in the Presence of Corpus Hemorrhagicum Cysts After Administration of Gonadotrophins to Rabbits.

S. R. M. Reynolds, Carnegie Institute of Washington, Department of Embryology, Baltimore 5, Md.

The terminal arterial supply of the ovary arises from the coils of one or two spiral arteries lying along the hilus of the ovary. This arrangement, it has been surmised, lowers arterial blood pressure within the ovary to near-osmotic levels, and equalizes it throughout the ovary. After injection of gonadotrophins, the coils become "paid out" evenly during the initial response of ovulation, luteinization, and generalized follicular growth. Accordingly, the spiral arrangement of the main arterial supply to the ovary permits ready adjustment to an increase in length of the ovary.

In a series of rabbits receiving gonadotrophins, and sacrificed at twenty-four hour intervals, a certain number showed fresh corpora hemorrhagica. Within the first twenty-four hours, the coils were evenly extended regardless of whether or not fresh small corpora hemorrhagica were present. It was observed that whenever large corpus hemorrhagicum cysts (measuring from about 2 to 3 millimeters across) were present two to six days after injection the cysts were distal to a region of disruption in the orderliness of the coils. Recent cysts have been seen on the ninth day which were associated with symmetrically arranged spiral arteries as well as with ones which were disrupted.

All specimens were injection-corrosion preparations in which vinylite was used as the injection mass. The preparations were studied from stereoscopic photographs enlarged two to three times.

4. Gonadal Stimulation Following the Administration of Antigonadotrophic Serum.

Herbert S. Kupperman, Roland K. Meyer, and J. C. Finerty. From the Zoological Laboratories, University of Wisconsin, Madison, Wisconsin, and the University of Georgial School of Medicine, Augusta, Georgia.

Antigonadotrophic serum obtained from rabbits injected with aqueous (unfractionated) extracts of sheep pituitary was administered to immature rats. The serum was injected for a period of 10 days to two groups of rats, one and ten days old, respectiveoy. Vaginal introitus occurred in the animals treated with antigonadotrophic serum 3–4 weeks prior to that in the littermate control. Patency of the vagina occurred, on an average, 10.9 days after cessation of antigonadotrophic therapy. Associated with the observed precocious sexual maturity there was marked ovarian and uterine stimulation over and above that seen in the control females. Basophilic changes were observed in the pituitary glands after the ten day treatment with antihormone. However, gonadotrophic assays performed at this time showed the pituitary of the antihormone-treated rats to contain less gonadstimulating substances than that of littermate controls. These data demonstrate that precocious sexual maturity, endogenous in origin, may be the result of morphological changes induced in the hypophysis of the injected animals. The data present evidence that the ovary of the sexually immature rat secretes a gonadal hormone, the absence of which will cause basophilism in the anterior pituitary.

5. Liver and Gonadal Changes Following the Administration of Carbon Tetrachloride to Male Rats and Female Guinea Pigs.

Boris Krichesky, S. J. Glass, E. Furlong, and M. Feiner. Department of Zoology, U.C.L.A.

Previous experimental and clinical observations suggest:

1. That a liver-gonadal syndrome may follow or be associated with sustained and extensive liver damage,
2. That the impairment of gonadal function may be the end result of hepatic failure to inactivate the steroid hormones, especially the estrogens and androgens.

To test this theory, further observations on this liver-gonadal relationship are being reported. It was found that the feeding of carbon tetrachloride to male rats and female guinea pigs was followed by hepatic cirrhosis and gonadal damage in both groups of animals. In the guinea pigs, estrogen excretion is strikingly increased for over 50 days to be followed by a drop within 60 days to pretreatment levels. The ovaries at the terminal phase of this esperiment show striking atrophy.

Whether these gonadal effects are a direct result of the liver damage and/or changes in pituitary gonadotrophic function is being investigated.

Lantern slides of the various morphologic changes observed in the sequence of progressive liver and sex organ damage will be shown.

6. Clinical Evaluation of Dienestrol, A Synthetic Estrogen.
A. E. Rakoff, K. E. Paschkis, and A. Cantarow. Jefferson Medical College and Hospital.

Dienestrol (4:4′ dihydroxy-γ-δ-diphenyl-β:δ-hexadinestrol was evaluated in 82 patients with various conditions in which estrogen therapy was indicated with reference to clinical improvement, objective response, and evidences of toxicity.

In 40 menopausal women dienestrol afforded excellent relief in every instance, the minimal dosage necessary ranging from 0.1 to 1.0 mgm. with an average of 0.43 mgm. daily. However, the average dosage required to improve the deficiency smear to a slight estrogen effect was 0.77 mgm. while to produce a marked estrogen effect required much higher dosages. Bleeding during the course of treatment or on withdrawal occurred only twice on therapeutic dosages and in two other cases with higher dosages. It was difficult to induce bleeding in younger women with amenorrhea even with high dosages. In 26 postpartum patients inhibition of lactation was readily obtained with dosages of 1.0 mgm. daily for 3 days and 0.5 mgm. thereafter for 1 week. These findings suggest that dienestrol has a relatively marked inhibiting effect on the pituitary.

No symptoms of intolerance were noted to dienestrol in any patient even in high dosage. In 25 patients treated for 4 to 12 months (average 6½ months) no evidences of toxicity were noted nor any significant changes in blood count, urinalysis, or liver function studies.

7. Diethylstilbestrol Dipalmitate in Aqueous Suspension.
S. Charles Freed, Department of Medicine, Mt. Zion Hospital, San Francisco, California.

We have demonstrated that there are at least two advantages in preparation of fat soluble crystalline sex hormones suspended in aqueous medium (estrone, stilbestrol and testosterone). These advantages are freedom from the allergic reactions of the usual oil vehicle and increased therapeutic effectiveness through a delay in absorption of the aqueous suspension over that of the oil solutions. Diethylstilbestrol dipalmitate, a slowly absorbed estrogen with prolonged action was suspended in an aqueous medium to determine whether this possessed the advantages over an oil solution. Five mg. of the material containing 1.6 gm. of the active component was administered every two weeks to menopausal patients according to the plan established for the other preparations. Eighty-six patients received 242 treatments. There appeared to be no appreciable difference in the beneficial effect of these patients with a comparable number of patients receiving an equal amount of the same material dissolved in oil. Either the oil solution is as slowly absorbed as the aqueous suspension or else there is no enhancement of effect by further slowing down the absorption of this preparation. The only advantage therefore of an aqueous suspension of diethylstilbestrol dipalmitate over that in oil is its freedom from local allergic reactions.

8. The Effect of Diethylstilbestrol Upon Alloxan Diabetes As Related to Food Intake in the Rat.

Dwight J. Ingle. The Research Laboratories, The Upjohn Company, Kalamazoo.

Diethylstilbestrol and other estrogens were found to be diabetogenic in the partially depancreatized, force-fed rat (Ingle, D. J., Endocrinol. 29: 838, 1941), whereas Janes and Dawson (Endocrinol. 38: 10, 1946) failed to observe a diabetogenic effect of diethylstilbestrol in alloxan-diabetic rats which ate *ad libitum*.

The present experiments show that the effect of diethylstilbestrol upon alloxan diabetes is determined by the intake of food. Male rats of the Sprague-Dawley strain (initial weight, 300 grams) were made diabetic by repeated intraperitoneal injections of 25 mgm. of alloxan. All of the animals were fed a fluid medium carbohydrate diet.

When diethylstilbestrol was administered to animals in which the food intake was kept constant by forced feeding, there was a striking exacerbation of the diabetes in every case, and the effect disappeared when the administration of the estrogen was stopped.

Those animals which ate *ad libitum* voluntarily consumed more food than was given by forced feeding, but when the estrogen was administered the food intake dropped sharply and the amount of urinary glucose was decreased significantly.

9. The Factor of Previous Treatment in Experimental Menstruation.

Doris H. Phelps. Department of Obstetrics and Gynecology, Vanderbilt University School of Medicine, Nashville, Tennessee.

It has been shown previously that the duration of experimentally induced menstruation can be influenced by a number of factors; among these are the intensity of hormonal stimulus, its composition and the duration of its action. This report deals with a hitherto undescribed factor of great importance, namely, the structure of the endometrial vascular bed at the beginning of the cycle.

To obtain information concerning mechanisms involved in the production of menstrual abnormalities, eight ovariectomized monkeys were injected with various combinations of the ovarian hormones. Endometrial vascular phenomena were observed by means of intraocular endometrial transplants and these observations were correlated wuth observations on uterine bleeding. One hundred seven cycles of uterine bleeding were produced, 1–13 per monkey. Analysis of the results of each experiment in relation to treatment administered in previous experiments in the same animal revealed that 1) permanent changes in the structure of the endometrial vascular bed may result from stimulation by the ovarian hormones and 2) the architecture of the endometrial vascular bed at the beginning of any given course of treatment with ovarian hormones exerts an important influence upon the uterine bleeding produced by that course of treatment.

10. Experimental Alteration of the Human Ovarian Cycle by Estrogen.

Willis E. Brown, J. T. Brandbury, and A. F. Jennings. University of Iowa, Department of Obstetrics and Gynecology, Iowa City, Iowa.

Patients were treated with stilbestrol at different phases of the cycle to determine its effect on menstrual rhythm and endometrial histology. When a single 20 mg. dose of stilbestrol was given orally in the early proliferative phase (4th to 10th day), the length of the interval was increased to an average of 38 days (range 32–48 days in 14 trials). Twelve untreated cycles in these patients averaged 29 days in length (range 24–35 days). Six of the treated cases experienced a very scant vaginal bleeding for a few days starting about a week after receiving the estrogen. The endometrium remained in a proliferative phase until about 10 days before the onset of bleeding and then it exhibited the normal secretory changes. There was one exception, a cycle of 33 days, in which the endometrium did not exhibit any secre-

tory changes, suggesting an anovulatory cycle. Under these conditions, a single large dose early in the cycle seems to delay ovulation about nine days.

Eight patients with dysmenorrhea were treated with oral stilbestrol starting the day after the cessation of menstruation. The dosage was 1 mg. daily for 10 days, 2 mg. daily for the second 10 days, and 3 mg. daily for the third 10 days. With this regimen the patients were relieved of their dysmenorrhea for three successive months and endometrial biopsies indicated that all three cycles had been anovulatory. Two other patients became so nauseated that they discontinued the treatment. One patient experienced a marked increase in weight, due to an edema which subsided quickly after stopping the medication. She then had an interval of 55 days before her next spontaneous menstrual period. Under these conditions, a continuous and increasing dosage for three weeks, it is possible to suppress the luteal phase for as long as three successive months.

When estrogen medication was started in the post-ovulatory phase of the cycle, 5 to 10 mg. of stilbestrol per day, there was no appreciable alteration of the luteal phase or any delay in the onset of menstruation. To date it has not been possible to prolong the life of the corpus luteum by estrogen therapy in the woman.

11. Studies on the Variations of Blood Gonadotrophins and Vaginal Smears During Pregnancy in Correlation with the Fetal Sex.

H. E. Nieburgs, H. S. Kupperman, and R. B. Greenblatt, Department of Endocrinology, University of Georgia, School of Medicine, Augusta, Ga.

The investigation of vaginal smears in 253 women during the 8th to 36th week of pregnancy revealed a progressive proliferation of the-vaginal epithelium due to increased estrogen activity. This is accompanied by increased glycogen deposition and massive desquamation of cells due to the action of progesterone. In addition to these progressive changes in the vaginal mucosa a number of specific smear types were observed. These were grouped into the mucoid-cornified, glycolytic and plasmolytic smears. There was no correlation of these smears with threatened abortions or toxemias. Where specific smear types were present the sex of the infant was predetermined with an accuracy of 85.4 per cent.

In a further group of pregnant women assays of blood gonadotrophins were carried out on 21–24 day old mice, estimating the FSH and LH fraction. The amount of FSH present was mainly constant, somewhat decreasing towards the later stages of pregnancy. (The determinations were made for dilution of serum up to 1:40). With the method employed the effect of LH was either totally absent or was present in dilutions up to 1:10 and rarely in dilutions of 1:20.

Data is presented on the relation of blood gonadotrophin levels to the sex of the fetus.

12. Oral Estrogen Therapy During Menopause.

Abbey David Seley, Deborah Baumgold, and Samuel Vernick. Gynecological Endocrine Department, Hospital for Joint Diseases, New York City.

Management of the menopausal state by oral estrogens alone was begun on July 1, 1943 in the Gynecological Endocrine clinic of the Hospital for Joint Diseases. For the past three and one half years not a single injection of any estrogenic substance has been given. Since oral estrogens are derived from various sources and/or have different chemical structures, a comparative clinical study of 90 unselected and consecutive menopause patients was initiated in order to evaluate the effectiveness of these substances. The purpose of this report is to indicate the relative value of the various estrogenic substances used in regard to: (1) ability to control the neurocirculatory disturbance characteristic of the menopause; (2) the rate of change of the vaginal smear; (3) the possibility of carcinogenic alteration when estrogens are used in doses sufficient to control the menopause as evidenced by endometrial biopsy and biopsy or curettage of the cervical stump; (4) the num-

ber and type of induced side reactions including bleeding; (5) the final results obtained by the oral route alone a) in the average case and b) in cases complicated by menopausal arthralgia, a number of which are found in this particular hospital.

13. Correlation of Basal Body Temperature Curves with Endometrial Biopsy.

A. R. Abarbanel, Department of Obstetrics and Gynecology, College of Medical Evangelists, Los Angeles, California.

Correlation studies of several huhdred endometrial biopsies with the basal body temperature curve for that cycle reveals that on the whole, the classically sharply defined diphasic B.B.T. curve is usually associated with a presumed ovulatory cycle as witnessed by successful conception or a fully developed secretory endometrium.

On the other hand, study of various aberrations revealed a secretory endometrium, especially of the so-called mixed type or immature progestational phase (day 19–22 of a 28 day cycle) when taken on the first day of menses. B.B.T. curves were either irregular or flat.

It is postulated that an immature progestational phase or a mixed type of endometrium do not necessarily represent ovulation but merely various degrees of theca luteinization without concomitant ovulation. In brief, a staircase phenomenon is postulated for the human, similar to that proposed for the macacus rhesus by Hartman.

14. A Rapid Method for the Determination of Urinary "17-Ketosteroids."

I. J. Drekter, Sidney Pearson, and Thomas H. McGavack, New York Medical College, Metropolitan Hospital Research Unit, Welfare Island, New York.

A method for the rapid clinical determination of urinary "17-ketosteroids" in volumes of urine as small as 10 ml. is described. Easily available, inexpensive laboratory equipment is used, and the hydrolysis extraction, and assay can be completed within four hours.

Optimum conditions for the hydrolysis and extraction of urine volumes from 10 ml. to 50 are given. Heating at 80° for 10 minutes gives the apparent maximum Zimmerman color producing substances.

The results of extraction with several solvents is given. Ethyl ether gives the best results. Recoveries are good. Normal value of 38 young men between ages of 18–30 by this method ranges from 10 to 30 with an average of 16.0 mg. excretion in 24 hours.

15. Further Studies on the Metabolism of Therapeutic Doses of the Natural Estrogens in Human Subjects.

Benjamin F. Stimmel and Clair L. Stealy, Rees-Stealy Medical Research Fund, Ltd., San Diego, Calif.

We have shown that our procedure for the chromatographic fractionation and photometric estimation of urinary estrogens can be used to study the metabolism of therapeutic doses (1–2 mg.) of the natural estrogens in human subjects. These studies were made on (a) a normal human female, (b) a bilaterally ovariectomized, hysterectomized human female, and (c) a bilaterally ovariectomized human female. The present report concerns similar estrogen studies on (a) a normal human male and (b) a bilaterally orchiectomized human male. These studies were made to establish a basis for work of a similar nature on human subjects with cancer of the prostate gland. In general, the human male metabolizes therapeutic doses of the natural estrogens in a manner similar to the bilaterally ovariectomized hysterectomized human female. Estriol apparently is not converted to estrone or estradiol, estrone and α-estradiol are partially interconvertible and the injection of either leads to the excretion of estriol also, and only a small fraction of the injection ma-

terial of any of the threee strogens is recovered in estrogenically active forms. The nature and amount of the estrogens excreted are profoundly influenced by (a) the vehicle in which the hormone is administered, (b) the route of administration, and (c) the nature of chemical conjugation of the administered hormone. The presence of the male gonads appears to influence to some degree the manner in which the therapeutic doses of the female hormones are metabolized. From these studies certain conclusions can be drawn regarding the type and amount of estrogenic substances which might prove satisfactory for further studies.

16. A Simple Quantitative Colorimetric Test for Estrogens.

Herman Cohen and Robert W. Bates, E. R. Squibb and Sons, New Brunswick, New Jersey.

The Kober test for estrogens and various modifications thereof, consist of two steps: 1) Initial heating with a small volume of H_2SO_4 mixed with a phenolic compound and 2) dilution with water or aqueous H_2SO_4 and reheating.

Absorption spectrum studies have shown that the presence of phenol, B-napthol, sulfonic acid or thiocol does not change the absorption curves from those obtained using H_2SO_4, alone, nor are the extinction coefficients appreciably different. The action of phenol is to quench the green fluorescence which affects the visual appearance of the red color but not the response of the photoelectric cell.

Based on these observations the following simple procedure, omitting the phenol reagent, has been developed.

0.4 cc. of an ethanol solution of estrogen is heated with 2.0 cc. of concentrated H_2SO_4 for three minutes in a boiling water bath. Remove tube from bath and add at once 8 cc. of 25% H_2SO_4 (by volume). Stir and heat the solution again for three minutes at 100° C. Cool in tap water and read the color density in a colorimeter using a 510 mμ filter. The color developed is stable at room temperature for four hours. Beer's law holds between the limits of 5 to 60 gamma of estrone.

The intensities of the color produced with estrone, estradiol and estriol differ, so that quantitative determination of each compound in a mixture requires fractionation.

17. Urinary Steroid Balance in Virilism and Hypogonadism.

W. T. Salter, Frances D. Humm, N. Jane Oesterling, and William W. Engstrom. Laboratories of Pharmacology and Toxicology of the Yale University School of Medicine, New Haven, Conn.

Microchemical determinations of estrogens and 17-ketosteroids have been applied to human urine in selected clinical cases. These methods are applicable to excretion intervals as short as 6 to 12 hours. The ratio of antithetical steroids (micrograms of total estrogen divided by milligrams of total 17-ketosteroids) is regularly under 1.0 in virile young adult males; in ovulating adult females it ranges from 2.0 to 100. In females with "adrenal metaplasia" the ratio is usually under 1.0, i.e., in the male range. Cushing's syndrome in females tends to show the same range, but the "corticoids" are usually high. Simple hirsutism, however, usually shows a normal female ratio.

In addition to the antithetical steroid ratio, the absolute magnitude of the respective steroid excretions must be considered. Both 17-ketosteroids and estrogens may be elevated proportionately in certain cases of female sterility and pseudohermaphroditism: so that the antithetical ratio is normal. In the delayed adolescence of males and in gynecomastia the antithetical ratio usually is below 1.0, even though 17-ketosteroids are low. In non-ovulating females the ratio remains continuously low.

The microchemical approach, lumping total estrogens, can be varied by preliminary separation of individual constituents if larger samples of urine are employed. For screening purposes in diagnosis and follow-up, such a separation has

not yielded spectacular results. The microchemical summation of estriol with the other follicular variants appears to be more advantageous than bio-assay both from a theoretical and from a technical standpoint.

18. The Metabolic Pathway of Estriol Production in the Organism.
Max N. Huffman and Arthur Grollman, from the Southwestern Medical College, Dallas, Texas.

The existence of a reversible equilibrium between estrone and α-estradiol and the conversion of the former to the latter in the human is well established. The pathway of the conversion of estrone to estriol is still, however, a matter of speculation. It is generally assumed that estrone is enolized and then hydrated to give estriol or that this hypothetical enol compound is formed directly by dehydrogenation of α-estradiol. A knowledge of the exact course of estrogen metabolism is important for it may lead to the production of more active naturally occurring estrogens as well as to better concepts of the possible role of these compounds in carcinogenesis and in the body economy in general.

An alternative hypothesis to account for the transformation of estrone to estriol is suggested; viz., that estrone is first converted to 16-keto-estrone which in turn is reduced first to 16-keto-α-estradiol and finally to estriol. One of us (M.N.H.) has prepared an epimer of estriol in which both C_{16} and C_{17} have α-configurations. This compound has been designated as iso-estriol-A. Reduction of 16-keto-estrone yields four possible stereoisomers, of which three have been prepared. We have determined the estrogenic activities of these compounds as well as of 16-keto-estrone prepared by partial synthesis from estrone. The results permit certain deductions regarding the probable pathway of estriol production in the organism.

19. Color Reactions of the Steroids.
Herbert Jaffe, Babette Solomon, and Robert H. Williams. Thorndike Memorial Laboratory, Second and Fourgh Medical Services (Harvard), Boston City Hospital, and the Department of Medicine, Harvard Medical School, Boston, Massachusetts.

A systematic survey of various color reactions of the steroids is being made with the principle objective of establishing and evaluating the structional characteristics essential for chromogenicity. Preliminary observations on thirty-seven steroids, comprising bile acids, sterols, androgens, estrogens and corticosteroids, and grouped according to progressive modifications in the nuclear ring substituents and structures, reveal that the following reactions can be associated with specific elements of the steroid structure:

Meta-Dinitrobenzene and Antimony-Trichloride Reactions. The m-dinitrobenzene reagent is specific for ketonic groups present either in the ring or in the side-chair. On the other hand, the presence of hydroxyl groups is essential for chromogenicity with the antimony-trichloride reagent, but the position of the alcoholic groups modifies the hue, intensity and rate of color development. Thus, for example, the hydroxycholanic acids yield a positive antimony reaction and a negative m-dinitrobenzene reaction, but the ketocholanic acids react conversely. Ketols vary in their reactivity towards each reagent, depending on the preponderant characteristics and the positions of the substituent hydroxyl and ketonic groups.

It is to be noted further that other polyvalent metals, viz. iron and aluminum (somewhat soluble in organic solvents as their chlorides) may replace antimony, effecting similar color changes.

20. Chemical Assay of Urine for Adrenocortical Hormones in Endocrine and Non-Endocrine Diseases.
William H. Daughaday, Herbert Jaffe and Robert H. Williams.

In determining the quantity of adrenocortical hormones in urine, we have adopted the principle proposed by Lowenstein of measuring the formaldehyde

liberated by the oxidation of urinary extracts with periodic acid. However, with Lowenstein's procedure certain substances in the urinary extracts interfere with the color reaction with chromotropic acid. To circumvent this difficulty, it is possible to distill the formaldehyde into a sulfite solution. A pure purple color is then obtained by the reaction with chromotropic acid and this is measured in an Evelyn photocolorimeter. We have used this method in 75 determinations. The results have been expressed as milligrams of adrenocortical hormone per 24 hours. Excretion by healthy adults has averaged 1.2 mgm./hours (range: 1–1.5 mgm./24 hours). Four patients with Addison's disease averaged 0.5 mgm./25 hours. Two cases of Cushing's syndrome were 2.8 and 23 mgm./24 hours. In 9 patients with exogenous obesity the excretion was normal, but in two determinations it was above normal. Low values were found in either hyper- or hypothyroidism. Hypopituitarism was associated with levels intermediate between Addison's disease and normal. Decreased excretion was also found in one patient with Waterhouse-Friderichsen syndrome and in one patient with scurvy.

21. Disappearance of Diabetes Mellitus Associated with Acromegaly Following Acute Mastoiditis and Basilar Meningitis.

T. P. Almy and Ephriam Shorr. Russell Sage Iustitute of Pathology, the Department of Medicine, Cornell University Medical College, and The New York Hospital, New York City.

Complete disappearance of diabetes mellitus was observed in a 40-year old male with acromegaly of 14 years' duration, the last 5 being complicated by diabetes and impotence. A 4+ glycosuria was regularly present despite 60 units protamine insulin daily. An oral glucose tolerance test gave the following blood sugar values: fasting, 250; 1 hour, 455; 2 hours, 555 and, 3 hours, 500 mg.%.

He then developed acute right mastoiditis and petrositis, basilar meningitis, retinal hemorrhages, visual field defects and ophthalmoplegia. On the 18th day of the illness, for which mastoidectomy was performed, the urine became sugar-free and fasting blood sugar and sugar tolerance curves had returned to normal levels. He has now remained free of diabetes for 5 years.

The BMR has fallen to −30% with no alteration, however, in serum cholesterol. There is no evidence of adrenal insufficiency. Impotence, associated with urinary 17-ketosteroid values of 2.5 mg., has persisted, except during androgenic therapy. Three years after this episode, the sella was found reduced in size.

The sudden disappearance of diabetes is attributed to partial degeneration of the anterior pituitary, during the acute infectious episode, with a consequent reduction in the elaboration of the diabetogenic principle.

22. True Hermaphroditism: Report of a Case with An Ovotestis, and Endocrine Studies.

John C. Weed (by invitation), A. Segaloff, Wm. Wiener (by invitation), and J. W. Douglas (by invitation). From the Divisions of Gynecology and Medicine, The Charity Hospital of Louisiana at New Orleans and the Alton Ochsner Medical Foundation, New Orleans, Louisiana.

A colored "female," age 36, exhibiting feminine bodily contours and feminine social adjustments, was found to have an enlarged clitoris, masculine facial hair distribution, and abnormal external genitalia. In a large hernial sac, an ovotestis was found associated with an atrophic uterus, left tube and ovary. Biopsy sections of the latter revealed normal ovarian stroma and a small Brenner tumor. Sections of the ovotestis which was completely removed, revealed normal ovarian stroma, definite testicular tubules without spermatogenesis, and large islands of interstitial cells. A rudimentary epididymis and fallopian tube were found in the hernial sac. Preoperative determinations of the gonadotrophic hormone and of 17-ketosteroids were made on 24 hour urine collections, and these were repeated postoperatively. The gonadotrophic hormones remained essentially unchanged in both series of

assays. The 17-ketosteroid determinations were 7.5 and 7.6 mg. per 24 hours preoperatively and from 3.8 to 5.6 mg. per 24 hours postoperatively.

23. Testosterone in a Case of Polyostotic Fibrous Dysplasia.

Rita S. Finkler and George M. Cohn. From the Endocrine Clinic and Service, Newark Beth Israel Hospital, Newark, New Jersey.

A case of polyostotic fibrous dysplasia observed over a period of several years is reported. The patient was referred for treatment to the Endocrine Service at the age of 13 after 4 years observation in other clinics. The most disturbing symptoms were progressive loss of vision in the right eye due to pressure of fibrous hyperplasia of the maxillary area upon the contents of the right orbit and progressive facial asymmetry. Since the disease is said to be self limiting at maturity, it was decided to attempt hastening maturity by the administration of testosterone propionate, because of the progressively failing vision. After one year of therapy, beginning June 1941, periodic examinations revealed gradual improvement in vision due to regression of bone pathology of the right orbit and a regression of the fibrous dysplasia in other bones of the body. At the last examination, in September 1946, there was almost complete recovery of vision in the right eye and regression of bone pathology in other parts of the body. It is felt that the use of testosterone propionate hastened maturity and the period of recession in this case since prior to treatment it was estimated that regression would not commence until after the age of 15. Lantern slides.

24. Bilateral Arrhenoblastoma Without Masculization, Adenoma Testiculaire of Pick.

Minnie B. Goldberg and Alice F. Maxwell, San Francisco.

Case Report

This report deals with a 19 year old girl presenting a paradoxical picture of primary amenorrhoea, tall eunuchoid build, *large breasts*, poorly developed external genitalia with rudimentary vagina, congenital absence of uterus and cervix, absent axillary and scant pubic hair and a high urinary gonadotrophin titer. The adnexae were not palpable.

Prior to performing a vaginal plastic operation surgical exploration of the pelvis was carried out with the surprising finding of bilateral, poorly developed gonadal structures composed of tissue grossly resembling adrenal cortex. This, on microscopic section, proved to be a highly differentiated type of arrhenoblastoma. Careful search revealed no evidence of ovarian tissue. Both tumors were removed. Bilateral rudimentary structures suggesting the unfused anlagen of uterus were also demonstrated.

The source of the estrogens responsible for the breast development remains a mystery. This case brings up many interesting questions concerning sex determination and differentiation involving problems in genetics, endocrinology and intersexuality.

Illustrated with photographs, photomicrographs and slides.

25. Social and Pyschological Readjustment of a Pseudohermaphrodite Under Endocrine Therapy.

Rita S. Finkler, Newark, New Jersey.

An intersex individual, aged 21 raised as a female, failed to develop either male or female secondary sex characteristics at puberty. The patient was asthenic and lacked endurance and energy, but excelled scholastically. The psychological status was of masculine pattern. He was fairly content until the age of 19, when he developed an emotional attachment for a girl and at the same time became aware of an enlarged clitoris. This discovery was somewhat of a shock and produced emotional conflicts resulting in symptoms of anxiety neurosis which lasted for over 2

years until a thorough investigation revealed the individual to be an "unfinished male." The investigations consisted of bio-assays, psychological analyses and exploratory laparotomy. Intensive substitution therapy with various testosterone compounds developed male secondary sex characteristics, and brought about a marked improvement in the patient's general health, energy, endurance and outlook on life. The patient was stimulated to still further scholastic and intellectual achievement. As long as therapy was continued, the patient's physical and psychological status continued to improve. Interruption in therapy for any appreciable length of time resulted in diminution of energy, endurance and mental alertness. Lantern slides.

26. Some Observations on the Utilization of a Liquid Chromatogram Technique in the Colorimetric Estimation of Urinary Pregnanediol.

Benjamin F. Stimmel. From the Rees-Stealy Medical Research Fund, Ltd., San Diego, California.

In our preparation of urinary phenolic residues for chromatographic fractionation of the estrogens, a neutral fraction of the ether soluble material is obtained. This fraction may be utilized for colorimetric (H_2SO_4) pregnanediol determinations in a procedure in which the chromatogram technique has been incorporated. Aqueous solutions of pure pregnanediol glucuronidate similarly treated have been found to yield two separate chromatographic fractions which respond to the color reagent. One of these is so weakly bound to the column of activated alumina that it appears in the initial benzene filtrate whereas the second (pregnanediol) requires much stronger eluting agents (2% methanol-benzene). It is hypothecated that a portion of the pregnanediol loses one or more of its hydroxyl groups during hydrolysis of the glucuronidate as has been shown to occur with alcoholic 17-ketosteroids under similar conditions. This partial destruction of the pregnanediol molecule is maximal during hydrolysis by autoclaving and minimal during simultaneous extraction and hydrolysis as described by Astwood *et al.* The use of the latter hydrolysis technique on our aqueous extracts of the butanol residues permits satisfactory colorimetric estimation of the pregnanediol content of both pregnancy and nonpregnancy urine. It appears that the method is sufficiently sensitive to detect pregnanediol excretion in a bilaterally ovariectomized human female following the injection of 10 mg. of progesterone. Some studies on pregnanediol and estrogen excretion by patients under simultaneous treatment with therapeutic doses of natural estrogens and progesterone will be presented.

27. The Oral Use of Crude Adrenal Cortex in the Stimulation of Growth of the Face, Particularly the Condyle of the Mandible.

Francis M. Pottenger, Jr., Monrovia, California.

This paper would consist of a complication of the results of a study of a group of patients who have been on adrenal cortical material for periods of two years or more. It would encompass some of our work over a fifteen year period.

Briefly stated, in patients taking the crude adrenal cortex regularly, we have noticed a definite increase in the development of the middle third of the face, but particularly in the length and thickness of the ramus of the mandible, and secondarily in the forward movement of the body of the mandible.

28. Chemical and Cytochemical Studies of the Rat's Adrenal Cortex Following the Administration of Pituitary Adrenocorticotrophic Hormone (ACTH)

Helen Wendler Deane (by invitation) and Grace E. Bergner (by invitation). (Introduced by E. W. Dempsey). Department of Anatomy and Medicine, Harvard Medical School, and the Medical Clinic, Peter Bent Brigham Hospital, Boston, Massachusetts.

Purified pituitary adrenocorticotrophic hormone (Armour), containing minimal

oxytocic activity, was injected into fasting adult male rats at varying doses and for varying periods of time up to twenty-four hours. Hypertrophy of the adrenal glands and atrophy of the thymus were observed with 2½ mg. of ACTH given every six hours for four doses. Adrenal cholesterol was decreased, and liver glycogen was increased.

Adrenals were fixed in formalin, and frozen sections were stained with sudan IV and Schiff's reagent, and unstained sections were studied for fluorescence and birefringence. Positive reactions by these methods characterize ketosteroids (Cf. Dempsey and Wislocki, *Physiol. Rev. 26*: 1,1946). In ACTH treated animals, birefringence and sudanophilia declined noticeably in the zona fasciculata of the adrenal cortex; whereas fluorescence and the Schiff reaction remained normally intense. Shorter treatment with 10 mg. ACTH or smaller doses of ACTH resulted in similar but less marked changes. These observations indicate that the zona fasciculata of the adrenal cortex is under pituitary control and secretes steroid hormones which influence thymus size and glycogen deposition in the liver.

29. Changes in Circulating Leukocytes Induced by Pituitary Adrenocorticotrophic Hormone (ACTH) in Man.

A. Gorman Hills (by invitation), Peter H. Forsham (by invitation), and Clement A. Finch (by invitation). Department of Medicine, Harvard Medical School, and the Medical Clinic, Peter Bent Brigham Hospital, Boston.

The effect of purified adrenocorticotrophin (Armour) upon blood elements was studied in normal subjects and in patients with Addison's disease. Intramuscular injections of 25 mg. of ACTH at 8:00 a.m. was preceded and followed four hours later by blood counts including direct eosinophil enumeration. Absolute polymorphonuclear, lymphocyte, and eosinophil counts four hours after ACTH were expressed as per cent of initial values.

In eight normal subjects ACTH administration induced an increase in neutrophils (90 per cent), a decrease in lymphocytes (40 per cent), and a decrease in eosinophils (78 per cent); whereas similar treatment in eight patients with Addison's disease was accompanied by only a slight increase in neutrophils (15 per cent) without a significant change in lymphocytes or eosionphils. Administration of 20 mg. of Compound F (17-hydroxycorticosterone) intramuscularly in four of these patients was followed by increased neutrophils (129 per cent), decreased lymphocytes (53 per cent), and decreased eosinophils (76 per cent). With Compound A (11-dehydrocorticosterone), 50 mg., these changes were minimal. No effect was observed with 11-desoxycorticosterone (10 to 20 mg.).

It follows that an increase in adrenal cortical hormone level is accompanied by significant hematological changes, viz. an increase in circulating neutrophils and a decrease in lymphocytes and eosinophils.

30. Results of Administration of Anterior Pituitary Adrenocorticotrophic Hormone to a Human Subject.

Harold L. Mason, Marschelle H. Powers, Edward H. Rynearson, Letizia C. Ciaramelli, Choh Hao Li, and Herbert M. Evans. Mayo Foundation, Rochester, Minnesota, and Institute of Experimental Biology, University of California, Berkeley, California.

A young woman was maintained on a constant diet while receiving intramuscularly 25 mg. of adrenocorticotrophic hormone daily for six days, 50 mg. for six days and 100 mg. for 11.5 days, given in five divided doses each day. Excretion of sodium, potassium, chloride, phosphorus, total nitrogen, creatine, creatinine, 17-ketosteroids and cortin-like substances was measured daily and that of pregnanediol, estrogens and gonadotrophic hormone at intervals. Levels of sodium, potassium, chloride, CO_2-combining power, cholesterol, ascorbic acid, protein, hemoglobin and alkaline phosphatase, the hematocrit and erythrocyte and lymphocyte counts were determined at intervals. During the first two periods the only signifi-

cant change was an increase in the excretion of cortin-like substances from an average control value of 0.180 mg. to average values of 0.255 and 0.387 mg., respectively. When 100 mg. per day was given the excretion of cortin-like substances increased to 1.44 mg. on the tenth day and the excretion of 17-ketosteroids increased from an average control value of 4.85 mg. to 15.5 mg. on the tenth and eleventh days. There was also a slightly increased nitrogen excretion, a decreased creatine excretion and a pronounced fall in hemoglobin. Other changes were minimal and of equivocal significance.

31. Metabolic Changes Following the Administration of Pituitary Adrenocorticotrophic Hormone (ACTH) in Man.

George W. Thorn, F. T. Garnet Prunty (by invitation), and Peter H. Forsham (by invitation). Department of Medicine, Harvard Medical School, and the Medical Clinic, Peter Brent Brigham Hospital, Boston.

Purified adrenocorticotrophin (Armour), 2 gamma giving a positive test by the method of Sayers and Sayers, with oxytocic activity of 0.12 units per mg., was administered to a male aged forty-six with partial reduction in pituitary function. On a constant dietary regimen the patient was given 10 mg. of ACTH in saline (pH 9) every six hours intramuscularly for six days (40 mg. daily). Peripheral vasoconstriction, bradycardia, and abdominal cramps occurred following each injection.

The following changes were observed:
Increased 11-oxy-steroid excretion (sevenfold)
Increased 17-ketosteroid excretion (fivefold)
Increased uric acid excretion (102 per cent)
Increased total nitrogen excretion (45 per cent)
Transitory increase in potassium and phosphorus excretion
Decreased sodium excretion (68 per cent)
No change in creatinine excretion
Increased fasting blood sugar level
Elevated glucose tolerance curve
Decreased serum inorganic phosphorus
Increased serum sodium and carbon dioxide combing power
Decreased plasma protein concentration
Decreased serum uric acid level
Increased polymorphonuclear leukocytes
Decreased lymphocytes and eosinophils

Control studies with pituitrin failed to show any of the above changes with the exception of hemadilution.

32. Urinary Uric Acid-Creatinine Ratio Following Administration of Pituitary Adrenocorticotrophic Hormone (ACTH) as a Simple Test for Adrenal Cortical Function.

Peter H. Forsham (by invitation), F. T. Garnet Prunty (by invitation), and George W. Thorn. Department of Medicine, Harvard Medical School, and the Medical Clinic, Peter Bent Brigham Hospital, Boston.

Earlier studies indicated that an increase in uric acid excretion with a rise in uric acid-creatinine ratio constituted one of the most consistent findings which followed the administration of the 11-oxy adrenal steroids (Compounds A and F) in man. The same changes have been found to accompany the administration of adrenocorticotrophin (Armour) in ten normal subjects, in five patients with miscellaneous diseases without evidence of adrenal insufficiency, and in two patients with anterior pituitary deficiency.

In contrast, it was noted that the maximum increase in uric acid-creatinine ratio following ACTH therapy in eight patients with Addison's disease did not exceed

the minimum increase observed in the control group; whereas the administration of either Compound F (20 mg.) or Compound A (50 mg.) induced a significant increase in the uric acid-creatinine ratio in these patients. The presence of renal impairment limits this test for adrenal cortical *reserve* function.

33. Further Studies on the Protective Power of Adrenal Preparations Against Bacterial Toxins.

Lena A. Lewis and Irvine H. Page. From the Research Division of the Cleveland Clinic Foundation, Cleveland, Ohio.

Determination of the relative protective power of adrenal extracts and steroids against typhoid vaccine in adrenalectomized rats has been shown to be a satisfactory method of estimating toxic protection power of hormones.

It seemed desirable to determine whether these substances would be effective in protecting adrenalectomized rats against other types of toxin and to compare the minimal lethal dose (M.L.D.) of the toxin as determined on normal and adrenalectomized rats as a possible means of evaluating the relative importance of adrenal activity in different infectious processes.

Streptococcus toxin, diphtheria toxin, crystalline tetanus toxin, and typhoid vaccine were tested. The streptococcus toxin was non-lethal to either normal or adrenalectomized rats in doses as large as 2.5 ml. of concentrated toxin. The M.L.D. of diphtheria toxin was 5 times as great for normal as adrenalectomized rats, while that of typhoid vaccine was 25 times as great for the normal. No difference could be demonstrated between the M.L.D. of crystalline tetanus toxin for normal and adrenalectomized rats, 60 units killing 100 per cent in both groups.

In adrenalectomized rats, larger doses of adrenal extract or 11-dehydrocorticosterone acetate were required to protect against 1.33 M.L.D. of diphtheria toxin than against 1.33 M.L.D. typhoid. No protective power of adrenal extract against tetanus toxin could be demonstrated. Studies to determine possible protective power of adrenal extract against bacterial peritonitis in mice are being carried out.

34. Effect of Testosterone upon the Excretion of Glycogenic Corticoids.

E. H. Venning and J. S. L. Browne. From the McGill University Clinic of the Royal Victoria Hospital, Montreal.

The excretion of urinary glycogenic corticoids was followed in a series of 8 individuals before and after the administration of testosterone proprionate or of methyl testosterone. Of these cases two were normal individuals, two were patients with Cushing's Syndrome, and the others suffered from hirsutism, diabetes, and one was a patient with lupus erythematosus disseminatus.

The administration of testosterone caused a lowering in the excretion of glycogenic corticoids in all cases with the exception of one of the patients with Cushing's Syndrome. In no case were the glycogenic corticoids depressed below the limit of normal values.

35. Hepato-Renal Factors in Circulatory Homeostasis: XVIII Relation of Adrenals to Formation of a Renal Vaso-excitor Principle.

B. W. Zweifach, Ephraim Shorr, Silvio Baez and S. Rosenfeld. From the Department of Medicine, Cornell University Medical College and The New York Hospital, New York City.

An inter-relationship has been demonstrated between the adrenals and the renal mechanism which elaborates the vaso-excitor principle (VEM) which we have detected in blood during the compensatory phase of shock and the acute phase of renal hypertension.

VEM is produced by kidneys from normal animals under reduced oxygen tensions *in vivo* and *in vitro*, and by kidneys of hypertensive dogs, both anaerobically and aerobically. It is detected by its potentiating effect on the response of the terminal mesentery blood vessels to epinephrine.

Following bilateral adrenalectomy (rats, rabbits, dogs) the renal capacity to form VEM became progressively impaired. Three precedures were employed: (1) adrenalectomy; (2) adrenalectomy plus high NaCl intake; (3) adrenalectomy plus NaCl and DCA (0.1 mg./kg. daily).

The mesenteric blood vessels of adrenalectomized rats exhibited a progressive unresponsiveness to epinephrine as adrenal insufficiency developed. *In vitro* incubation of kidney slices from adrenalectomized animals revealed progressive impairment of VEM formation even in animals maintained on high salt intakes. Kidneys removed from adrenalectomized animals maintained on NaCl and DCA for 10-15 days postoperatively behaved like normal kidneys in respect to VEM formation.

36. The Role of the Adrenal Cortex in Protein Catabolism Following Trauma.

C. G. Toby and R. L. Noble (introduced by J. B. Collip). Research Institute of Endocrinology, McGill University, Montreal, Canada.

Trauma (limb clamping) in the rat caused a marked excretion of urinary nitrogen. This was not increased by repeated cortin administration.

Nitrogen excretion in the normal rat was only moderately increased after treatment with a total dose of cortical extract equal to 240 dog units administered over 8 hours.

Nitrogen excretion in the adrenalectomized rat varied depending on the time after operation. It was slightly elevated by 4 days, and had returned to normal after two weeks.

Trauma when applied to the adrenalectomized rat did not cause any increased loss of urinary nitrogen.

Suitable treatment of traumatized rats with cortical extract allowed a practically normal response in nitrogen excretion to occur. The same treatment did not cause increased nitrogen loss in untraumatized adrenalectomized rats. Desoxicorticosterone acetate or hypertonic saline therapy were ineffective.

The experimental results indicate that following trauma, secretion of cortin is not responsible for the increased loss of nitrogen. On the other hand, nitrogen loss does not occur in the absence of the adrenal glands unless replacement therapy with cortin is administered. Adrenal cortical secretion is apparently necessary for the mechanism concerned to operate normally although such secretion per se is not responsible for the increased protein catabolism.

37. A New Hormone of the Adrenal Cortex.

Frank A. Hartman, Katharine A. Brownell and Jonathan S. Thatcher. Department of Physiology, The Ohio State University, Columbus.

The movement of fat from the reserves of the liver during inanition depends upon the adrenal cortex since it fails to occur if the latter is destroyed. The substance responsible, which we propose to call the "fat factor," has been separated from the sodium and carbohydrate factors by chromatographic adsorption. It causes deposition of fat in the liver of an adrenalectomized animal during starvation, while the other factors do not. Likewise, the crystalline compounds, corticosterone, 11-dehydrocorticosterone and 11-dehydro-17-hydroxycorticosterone, are unable to do this. Further evidence that the carbohydrate and fat factors are distinct has been obtained from animals whose adrenals have been enucleated. The output of these factors reaches a point above normal one or more weeks after enucleation. However, the maximum increase for the fat and carbohydrate factors develops at widely different times.

38. Studies on Obesity.

Robert H. Williams, William H. Daughaday, Walter F. Rogers, Jr., Samuel P. Asper, Jr., and Beverly Towery. Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard) Boston City Hospital, and the Department of Medicine, Harvard Medical School, Boston, Massachusetts.

Since obesity is often of major consequence and is not, on the whole, satisfactorily controlled, we have used recently available methods in the reinvestigation of certain phases of this aged problem.

The protein-bound iodine of the plasma, determined in 24 patients with marked obesity but with basal metabolic rates above −7 per cent, was found to be normal in 33 per cent, but in 67 per cent it might be considered subnormal, varying from 1.3 to 4.4 gamma per 100 cc. of plasma, with an average of 3.3 gamma. None of the patients had clear-cut clinical evidence of hypothyroidism and none had a goiter.

The excretion of ketosteroids in the urine was subnormal in 9 of 13 patients tested. The "corticosterone" excretion was found to be essentially normal. The glucose-insulin tolerance test yielded evidence of resistance to insulin in each of 8 patients.

Several weeks after removing each of the endocrine glands of rats, estimations of the "neutral fat" of the carcass showed no appreciable increase in the fat content. Moreover, no striking fat deposition resulted from the administration of large doses of the hormones in common use.

Over 100 markedly obese individuals were treated with from 1 to 7 compounds including 2-amino-6-methylheptane, dextroamphetamine, 1-cyclohexyl-2-aminopropane, p-hydroxy-alpha-methylphenethylamine hydrobromide, laevo-amphetamine, and d-desoxyephedrine hydrochloride. Of these compounds, the first two were the most satisfactory. The anti-obesity effect was apparently due to the decreased hunger and the increased exercise which the drugs produced.

39. Pellet Therapy with Desoxycorticosterone Acetate in Adrenal Cortical Insufficiency.

George F. Koepf and (by invitation) Raymond Kibler. Departments of Medicine and Physiology University of Buffalo, School of Medicine.

During the five year period (1941-1946) thirty-two patients with adrenal cortical insufficiency were treated with subcutaneously implanted pellets of desoxycorticosterone acetate (Ciba). Although sodium chloride in addition to the normal dietary intake was given during an assay period in which the daily dose of D.O.C.A. in oil was being ascertained, supplementary sodium chloride was seldom found necessary after implantation. Several patients were given adrenal cortical extracts during episodes of acute infections, but in only two cases was it necessary to supplement the therapy for prolonged periods because of severe, repeated attacks of hypoglycemia.

Data to aid in determining the number of pellets was obtained and may be summarized as follows:

(1) The average daily hormone absorption from a 125 mg. pellet (Ciba) by a human is 0.35 mg.

(2) Approximately 60% of the daily dose of D.O.C.A. in oil is required if the D.O.C.A. is given in pellet form.

(3) To determine the number of pellets of D.O.C.A. to implant the following formula is useful:

$$\text{pellets needed} = \frac{\text{daily dose D.O.C.A. in oil} \times 0.60}{0.35}$$

(4) The average duration of each implant was 334 days.

Eighteen of the thirty-two patients were diagnosed as non-specific adrenal cortical insufficiency, and two (11%) of these had died. All of the nine patients with tuberculosis of the adrenal cortex have died, however, some prolongation of life in most of these cases was noted. One (20%) of the five patients with adrenal cortical insufficiency secondary to panhypopituitarism has died.

40. Relationship of Sex Steroids to the Adrenal Glands of Hamsters and Rats.

Herbert S. Kupperman and Robert B. Greenblatt.

Sex dimorphism in the size of the adrenal has been reported in the rodents with the female animal exhibiting the heavier adrenal. However, data in the golden hamster revealed a reversal of this sex dimorphism whereby the adrenal of the male hamster was larger than that of the female. Autopsy performed on animals 15 to 600 days of age showed that the adrenal weights were comparable in both sexes until the 30th day of life at which time a sex difference became evident. The adrenal weight in both the male and female animals plateaued at 100 days of age with the weight of the male adrenals recorded at 65% greater than those of the female. The increase in size of the male adrenal coincided with the beginning of androgenic activity as evidenced by the appearance of dimorphic pigmentation in the male and the period of rapid growth of the prostate and seminal vesicles. Administration of sex hormones to castrate and normal male and female hamsters failed to demonstrate a specific action of the sex steroids. The effects of estrogen and progesterone upon the adrenal glands of albino rats were noted after estrogen administration. With the doses employed no hypertrophy of the adrenals was noted after estrogen administration. There was a variation in response to progesterone. The results, together with the morphological findings, are described and discussed in the light of the adrenal-gonad-pituitary relationship.

41. Correlations of Biochemical and Histological Changes in the Adrenal Cortex in Various Types of Disease.

Walter F. Rogers, Jr. and Robert H. Williams. Thorndike Memorial Laboratory, Second and Fourth Medical Services Harvard, Boston City Hospital, and the Department of Medicine, Harvard Medical School, Boston, Massachusetts.

Certain biochemical and physiological changes in the adrenal glands of 103 (selected) patients dying of a wide variety of diseases were compared with the glands of normal individuals who exprienced instantaneous or sudden death. The cholesterol content of the adrenal glands was compared with observations made of the tissue with polarized and ultraviolet light. Comparisons were also made of tissues stained with phenylhydrazine, Sudan IV, and phloxine-methylene blue. It is concluded that a close correlation exists in each of these methods of study and while they give an indication of the total amount and distribution of the lipoid deposition in the adrenal cortex, they do not demonstrate the amounts of adrenal hormones present, but they can be used to indicate the functional reactivity of the adrenal cortices in various types of disease.

42. The Use of Hypertonic Saline Infusions in the Differential Diagnosis of Diabetes Insipidus and Psychogenic Polydipsia.

Anne C. Carter and Jacob Robbins (introduced by Ephriam Shorr). From the Department of Medicine, Cornell University Medical College and The New York Hospital, New York City.

Hickey and Hare (*J. Clin. Investigation 23:* 768, 1944) have shown in animals and in a small series of normal human subjects that, after adequate hydration, infusion of 2.5% NaCl (0.25 cc./kg./min. in man) result in reduction in urine flow attributable to an antidiuretic factor of neurohypophyseal origin. This effect was absent in experimental and in two cases of human diabetes insipidus.

Additional evidence of the value of this procedure for differentiating diabetes insipidus from psychogenic polydipsia is provided by studies on a larger series of human subjects, using urine flow as the index. Nine normal hydrated subjects showed a marked reduction in urine flow during infusion of 2.5% NaCl. This reduction was found not to be due to spontaneous decrease in water diuresis nor to saline per se, and could be prevented by overhydration.

Of ten patients with polyuria, two showed reduction in urine flow during infusion and their subsequent spontaneous decrease in urine volume established the psychogenic basis for the polydipsia. The remainder exhibited sustained diuresis during the infusion and a prompt reduction in urine flow following 0.1U Pitressin intravenously indicating true diabetes insipidus. In the absence of renal disease, this test provides for differentiation between psychogenic polydipsia and diabetes insipidus.

43. Effect of Aqueous Testicular Extracts on Growth and Development of Spontaneous Mammary Tumors in the Aging Bitch.

F. X. Gassner. Colorado Agricultural Experimental Station, Fort Collins, Colorado.

An aqueous testicular extract of fresh beef testes, devoid of lipoids and essentially so of protein, was assayed for androgenic properties in intact and castrated male rats, and for estrogenic potency in castrated female rats. Each cc. of the extract represented 10 Gm. of fresh testes.

The sex organs of castrates of both sexes failed to show any response. The seminal vesicles, prostates and testes of the intact males were reduced in weight up to 46 per cent.

Nine aging female dogs with spontaneous mammary tumors received intramuscularly 10 cc. aqueous testicular extract weekly for up to eight weeks. Biopsies were taken at the start and at the end of treatment. Changes in the size of the tumors were photographically recorded weekly. In seven dogs the rapid growth of the tumors was not only arrested but their size was reduced as much as 86 per cent. The initial biopsies showed the presence of adenocarcinomas grades 1 to 3. After 6 weeks treatment extensive hyalinization of the stroma and necrosis and reduction of the tumorous parenchyma were evident. Decided improvement in behavior and well-being of the dogs was noted.

Gynecomastia in one intact male dog and in one castrated male cat subsided after 4 weeks treatment. The probable mechanism involved is discussed.

44. Conjunctival and Corneal Lesions in Hypercalcemia.

John Eager Howard and Frank B. Walsh. From the Departments of Medicine and Ophthalmology, The Johns Hopkins University and Hospital, Baltimore, Maryland.

In the past few years we have seen, in approximately 50 per cent of our patients with hypercalcemia, distinctive phenomena in the eyes which are believed to be of diagnostic importance and theoretical interest. Similar lesions have been observed by colleagues at the Massachusetts General Hospital. The crystals cannot be identified with the naked eye so that examination with the slit lamp is necessary.

In the deep conjunctiva of the palpebral fissure area are small glass-like particles. There is likely to be some redness, but this may not be pronounced; and there may or may not be complaint of conjunctival irritation. The crystals may be numerous or few. In one instance here and in several instances at the Massachusetts General Hospital, the peripheral superficial layers of the cornea have been involved presenting an appearance resembling band-shaped keratitis. Biopsy of one case was studied at the Johns Hopkins University and the crystals reported to be calcium phosphate. The crystals are clear and not at all like the grayish patches sometimes seen extending superficially over a pterygium. The lesions have been seen by us in

instances of hyperparathyroidism and vitamin D poisoning. In some instances the deposits have disappeared after the metabolic disturbance had been corrected.

Why the conjunctival and corneal membranes should be so involved is not clear. The factor common to our cases seems to be hypercalcemia, the serum phosphorus levels having been normal, high and low. Search for such lesions by ophthalmologists may uncover cases with hypercalcemia which would have otherwise been overlooked by the internist.

45. A Secondary Sexual Character That Develops in An Organ Common to Both Sexes But Normally Only in Men. With a Discussion of the Relation of This Character to Endocrine Stimulation.

James B. Hamilton. Long Island College of Medicine, Brooklyn, New York.

In organs present in both sexes only relative differences in secondary sex characters have been described. That is, a character is developed to a greater extent or occurs more commonly in one sex. Some interest attaches, therefore, to a masculine trait which distinguishes *absolutely* between normal men and women. This character is the growth of terminal (coarse) hairs on the external ear.

Three factors control development of this trait: ageing, endocrine stimulation (apparently androgenic) and genetic predisposition. Large auricular hairs grow in Caucasian men after the 24th year and increase in frequency and coarseness until by the 55th year 75 per cent of men exhibit this character (229 men studied). Terminal hairs were not observed in a series of 105 women and 50 eunuchs. Auricular hairs appeared in three of five eunuchs receiving androgenic treatment and in three of five women with pronounced virilism.

Pronounced hairiness depends more upon age and inheritance than any exceptional degree of androgenic stimulation. No terminal auricular hairs were observed in undoubtedly virile men among 54 American Indians, 84 Chinese and certain Caucasian families. Titers of urinary steroids in 57 normal Caucasian men bore no quantitative relationship to the amount of auricular hair.

46. The Use of Methyl Testosterone and Testosterone Propionate in Premature Infants

E. Kost Shelton and Jerome S. Mark. From the University of Southern California and The Shelton Clinic, Los Angeles, California.

Following a preliminary report by E. Kost Shelton, M.D., and Arthur E. Varden, M.D., before the Association of Internal Secretions, Saturday, June 29, 1946, the experimental use of testosterone, in various forms, on premature infants was continued at the County Hospital in Los Angeles.

Only premature babies below two thousand grams were employed in this study. Every third child was given methyl testosterone by mouth, every third child testosterone propionate by hypodermic, and every third child given nothing by way of specific treatment. The basic care of all these children was the same.

While all of the data are not yet available and the work continues, there seems to be a significant difference in 1) survival, and 2) weight increase in those children treated by testosterone preparations over those not so treated. The results continue to be promising.

New methods of administration of the hormones have been divised.

47. Equine Pituitary Gonadotrophin and Antihormone Formation.

James H. Leathem and A. E. Rakoff. From the Bureau of Biological Research, Rutgers University, New Brunswick, N. J., and the Department of Obstetrics and Gynecology and the Endocrine Division, Jefferson Medical College and Hospital, Philadelphia.

Equine pituitary gonadotrophin (Squibb) has been studied to evaluate it in terms of antihormone formation in cases subjected to somewhat extended therapy. Thirteen women suffering from anovulatory sterility or functional menstrual dis-

orders and four hypogonadal males have been studied for antihormone formation. All cases exhibited diminished urinary gonadotrophins. In general, the females received 50 units twice weekly during two weeks of each month over a 2-5 month period. The males received 50 units twice weekly for 3-5 months. Antihormones against equine pituitary developed in three women all of whom had been treated for three months and in three males in 3-4 months of therapy. No inhibitory substances existed prior to therapy. Antihormones against equine pituitary were also inhibitory against pregnant mare serum gonadotrophin. However, two cases in which the serum lacked anti-equine pituitary substances were able to inhibit pregnant mare serum.

Cases exhibiting a prompt response to equine pituitary were not studied for antihormone formation but of those here presented the cases of functional menstrual disorders exhibited a good response, the males a moderate response and 3 of 7 cases of anovulatory sterility exhibited improved temperature charts.

Equine pituitary gonadotrophin appears to have a distinct stimulating action on the human gonad but also elicits antihormone formation.

48. Studies in Cases of Pituitary Tumors.

K. E. Paschkis, A. Cantarow and A. E. Rakoff. Jefferson Medical College and Hospital, Philadelphia.

Studies are reported on a number of cases of chromophobe adenomas and of parapituitary tumors such as craniopharyngeomas.

The picture of hypopituitarism includes the effects of lack of gonadotrophin-, thyrotrophin- and adrenocorticotrophin secretion. The resulting deficiencies were studied by appropriate methods, in a number of cases both before and after operation.

In insulin tolerance tests "hypoglycemia-irresponsiveness" with prolonged severe hypoglycemia was observed with conspicuous absence of clinical manifestations usually present with severe prolonged hypoglycemia.

Impairment of salt metabolism was present in a number of cases. This indicates that, at least in the human, adrenocorticotrophic regulation of the adrenal cortex includes salt-water-metabolism contrary to the claim that the latter function is independent of pituitary regulation.

In one case of pituitary tumor the presence of which was indicated by X-ray of the sella and by visual field examination, there were hypogonadism (absent gonadotrophin excretion, amenorrhoea, atrophic endometrium) and lactating breasts. No other functional abnormalities were present. This patient has not as yet been operated upon, and the nature of the tumor is therefore not known.

49. Carbohydrate Appetite of Normal and Hyperthyroid Rats As Determined by the Taste-Threshold Method.

Curt P. Richter. Psychobiological Laboratory, The Johns Hopkins Hospital Baltimore, Maryland.

Fifty-four normal and 28 hyperthyroid rats were tested with the taste-threshold method for their appetites for 6 common sugars: maltose, glucose, sucrose, fructose, lactose and galactose. Hyperthyroidism did not change the taste thresholds nor the maximum preference concentrations of these 6 sugars. It did produce a great increase in the appetite for the sugars. The hyperthyroid rats took about twice as much of each of the sugars as did the normal animals. Further, they definitely preferred certain concentrations of the sugars to the stock diet.

Both normal and hyperthyroid rats showed the greatest appetite for the corn sugars, maltose and glucose, the smallest appetite for the milk sugars, lactose and galactose, and intermediate appetites for the cane sugars, sucrose and fructose. The results indicated that the appetites varied directly in proportion to the abilty of the rats to assimilate the sugars. It was concluded that hyperthyroidism produces a great increase in the ability of the rats to assimilate carbohydrates.

50. The Effect of Hypothyroidism on Menstruation.

W. O. Thompson, P. K. Thompson and E. M. Jeppson.

A study of spontaneous myxedema developing in adult life shows that it may produce an increase, a decrease, or a complete absence of menstrual flow. Myxedema developing in adult life as a result of subtotal thyroidectomy often produces an increase in flow but does not seem to produce a decrease. The increase in menstrual bleeding with hypothyroidism may at times be very great, simulating an incomplete abortion and producing a secondary anemia.

Regardless of the type of menstrual disturbance, the administration of thyroid in a suitable dose may correct it. However, when there is a complete absence of menstruation, it may fail to return in some instances following the administration of desiccated thyroid even though the patient is still below the age of the menopause.

The hypothesis that excessive bleeding is the result of primary hypothyroidism while oligomenorrhea and amenorrhea are associated with hypothyroidism secondary to hypopituitarism is not supported by the fact that both types of menstrual irregularity in patients with myxedema may be corrected by the administration of thyroid. The failure of amenorrhea to disappear with thyroid feeding may point to its pituitary origin.

51. Twelve Cases of Metastatic Thyroid Carcinoma Studied with Radioactive Iodine.

S. M. Seidlin, E. Oshry and A. A. Yalow. Montefiore Hospital, New York, N. Y.

After the administration of tracer doses of radioactive iodine to twelve patients with thyroid carcinoma, the metastases were studied for uptake of the isotope by means of the Geiger-Mueller counter. Six cases were positive (metastases showed uptake of radioiodine), three were negative and three questionable. The radioautograph were positive in seven, negative in the three and questionable in two. The possible correlation of histological structure of the tumors and radioiodine uptake was attempted. The effect of pretreatment with thyrotropic hormone and thiouracil on radioiodine uptake by the metastases was also studied. Radioiodine concentration in the blood and urinary excretions were carried out in all cases. All "positive" cases received or are receiving therapeutic doses of radioactive iodine.

52. Goiter on an Iodine-Free Diet Grown by Hydroponics, and Excluding Any Goiter Noxa.

J. F. McClendon and Wm. C. Foster. Hahnemann Medical College, Philadelphia.

In order to produce an iodine-free diet and exclude a goiter noxa we grew a diet by hydroponics in a disinfected greenhouse with disinfected water and chemicals in a goiter-free region. Air was pumped through a carbon filter. Six litter-mate rats from a colony that had been goiter-free for 6 years were put at weaning on the diet of 40 per cent sunflower seed, 2.8 per cent soy beans, 40 per cent sucrose, 0.8 per cent NaCl and 16.4 per cent corn oil. Three of the rats were given water redistilled from alkali to drink and at the end of 73 days had goiters weighing 39, 42 and 41 mg. per 100 gms. body weight. The other 3 rats were given water containing 10 parts per million of iodine and had normal thyroids weighing 10 mg. per 100 gms. body weight. Since the rat cages were enclosed in a cellophane covered frame and no goitrous animals or humans had access to them we believe that so called goiter noxa is excluded. The goiters were 4 times as large as normal thyroids and twice as large as goiters in rats from a goitrous colony fed in a goiter region on a very low iodine diet.

53. Mitotic Activity and Wound Healing in the Corneal Epithelium of Rats Treated with Thiouracil.

Walter Fleischmann and Alfred Breckler. Clinical Physiology Section, Medical Division, Edgewood Arsenal, Maryland, and Department of Pediatrics, Johns Hopkins University, Baltimore, Maryland.

The corneal epithelium of the rat has been shown to be a suitable test object for mitotic and wound healing activity. (Buschke, Friedenwald and Fleischmann, *Bull. Johns Hopkins Hosp. 73:* 143, 1943; Friedenwald and Buschke, *J. Cell. and Comp. Physiol. 23:* 95, 1944). We have compared mitotic counts in flat preparations of corneae of rats treated with thiouracil with those of litter-mate controls. A number of animals were given 5 mg. of colchicine per kg. of body weight 4 hours before killing them, others were killed without previous treatment with colchicine. The number of mitoses as estimated from the colchicine experiments was significantly reduced in the rats treated with thiouracil. In the experiments without colchicine the counts were not reduced in the rats treated with thiouracil. This indicates that not only the onset of mitosis, but the duration of the mitotic cycle is slowed down in thiouracil treated rats. Superficial wounds of the cornea heal in the same time in hypothyroid rats as in normal controls.

54. Clinical Manifestations in Forty Cases of Myxedema.

David Schwimmer, Mildred Vogel, and Thomas H. McGavack. From the New York Medical College, Metropolitan Hospital Research Unit.

Data are presented on forty cases of myxedema seen during the past seven years in both hospital and office practice. Observation has varied from 12 hour antemortem to over five years.

Stress is laid on the presenting clinical manifestations, chiefly as they concern proper diagnosis. It is felt that far too often the diagnosis of myxedema is missed primarily because it is not entertained as a possibility. An important contributing factor in such failures of recognition of the disease is that the diagnosis generally is based on the classical findings of obesity, dry skin, coarse hair, hoarse voice, apathy, etc.

While it is true that these classical manifestations are unmistakable, such standard findings may be absent. In 20–30%, the myxedematous status was masked by symptomatology of cardiac difficulties. "Nervousness," rather than apathy and complacence, was prominent in over 60%.

The cases presented are divided into four groups:

I. Those with classical symptomatology
II. Those with predominantly cardiac manifestations
  a. cardiac cymptoms without congestive failure
  b. cardiac symptoms with congestive failure
  c. cases of coronary occlusion
III. Those with atypical symptomatology
IV. Those without symptoms

Emphasis is placed on the fact that cardiac failure may raise the basal metabolic rate to normal or above; also, that the serum cholesterol levels may be unexpectedly normal, especially with chronic passive congestion of the liver incident to right heart failure.

55. Thiouracil in the Treatment of Hyperthyroidism Complicating Pregnancy and Its Effects on the Fetus.

M. James Whitelaw. From the Department of Obstetrics and Gynecology, Southwestern Medical College.

The relative infrequency of hyperthyroidism as a complication of pregnancy is discussed giving the statistical figures from both European and American sources. A severe hyperthyroid pregnant white female in the 26th week of gestation was

treated with thiouracil up to and through the puerperium. She presented the classical clinical as well as laboratory findings characteristic of Graves' disease. Her BMR was +65. Under Thiouracil therapy .4 gram per day given in divided doses plus general supportive measures, she was symptom free at the time of her delivery. Her BMR having fallen within normal range as had her pulse, blood chemistry and circulation time. An anencephalic monster was delivered which died 6 hours postpartum. Autopsy revealed a thyroid gland that appeared both microscopically and histologically normal. The weight and iodine content of the thyroid were both well within normal limits.

56. The Permanency of Alloxan Diabetes and the Structure of the Pancreatic Islets Following Certain Experimental Procedures.

Ralph G. Janes, Department of Anatomy, College of Medicine, State University of Iowa, Iowa City.

When alloxan is given to the rat either subcutaneously, intraperitoneally, or intrasplenically, there is considerable variation in the severity of the resulting diabetes. In mildly diabetic animals (blood sugar up to 250 mg. % and urine volume below 100 cc. daily) the diabetic symptoms usually disappear in 4–6 weeks. In severely diabetic rats (blood sugar levels up to 900 mg. % and urine volumes up to 300 cc. daily) the diabetes remains severe until death. Although it is not always possible to correlate the severity of the diabetes with the amount of islet damage, in general the more severe the diabetes, the more extensive is the islet destruction. When suitable quantities of alloxan are administered the islets lose their brilliant staining qualities, cell boundaries become indistinct, and many of the Beta cells exhibit necrotic changes. Furthermore, islet cell nuclei become more abundant at the periphery, resulting either from shrinkage of the cytoplasm of certain islet cells or perhaps from cell division. In 3–4 weeks following the injection of alloxan, some of the Beta cells which are not destroyed regain their staining qualities and connective tissue replaces those which are destroyed. In an attempt to modify further the islets following this period, the animals were subjected to several procedures which have been shown to alter carbohydrate metabolism, namely, diets high in niacin, diets with large amounts of thiamine, high fat diets and thiamine deficient diets. No further alteration in the islets has been noted following these procedures.

ASSOCIATION NOTICE

SQUIBB AND CIBA AWARDS
ASSOCIATION FOR THE STUDY OF INTERNAL SECRETIONS

The Association for the Study of Internal Secretions has just announced that the award provided by E. R. Squibb and Sons has been conferred on Doctors Carl F. and Gerty T. Cori of Washington University, the award furnished by the Ciba Pharmaceutical Company has been made to Doctor Choh Hao Li of the University of California, and the newly established fellowship of Ayerst, McKenna and Harrison, Limited has been given to Doctor Samuel Dvoskin of Columbia University.

The Squibb Award to Dr. and Mrs. Cori was based on major contributions which they have made on the action of adrenalin, insulin, and the anterior pituitary and adrenal cortical hormones concerned with metabolism. They have described the metabolic transformations of glycogen, glucose and lactic acid in the liver, blood and muscle under the influence of adrenalin and insulin. They have isolated the enzymes concerned in glycogen formation and by this means have accomplished the synthesis of glycogen in vitro. Their more recent studies have indicated that insulin and the hormones of the anterior pituitary produce at least some of their effects by controlling the rate of one of the enzymatic steps concerned with carbohydrate utilization.

Dr. Carl F. Cori was born in 1896. He received the degree of Doctor of Medicine from the German University, Prague, Austria in 1920. He served as assistant in the medical clinics in Prague and Vienna and as assistant in Pharmacology at Graz. He was biochemist at the State Institute for the Study of Malignant Diseases, Buffalo, 1922–30, and Assistant Professor of Physiology at Buffalo 1930–31. From 1931 to 1941 he was Professor of Pharmacology, and from 1941 to 1946, Professor of Pharmacology and Biochemistry at Washington University. Since 1946 he has been Professor and Chairman of the Department of Biochemistry at Washington University. Dr. Gerty T. Cori was born in 1896 and received the degree of Doctor of Medicine at Prague in 1920. She was assistant pathologist and biochemist at the State Institute for the Study of Malignant Diseases in Buffalo from 1922 to 1931. Since 1931 she has been Research Associate in Pharmacology and Biochemistry at Washington University.

The Ciba Award to Doctor Li was given for his significant contributions in the separation and study of hormones of protein nature and the isolation of the adrenocorticotrophic and growth hormones in homogeneous and highly purified states. Dr. Li was born in Canton, China on April 21, 1912. He received the degree of Bachelor of Science from Nanking University in 1933 and the degree of Doctor of Philosophy from the University of California in 1938. From 1933 to 1935 he was an instructor at Nanking University. Since that time he has served at the University of California as Research Associate, 1938–1942, as Assistant Professor, 1942–1946, and since 1946 as Assosiate Professor of Experimental Biology.

Doctor Samuel Dvoskin was named to receive the first Ayerst, McKenna and Harrison Fellowship. He received both the Ph.D. and M.D. degrees from Columbia University in 1945 and in his studies on the testis and thyroid has shown unusual promise as an investigator. He will continue his work on the thyroid gland and thyroid hormone assays under Dr. Robert Loeb.

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